On Aphids, their Host Plants and Speciation

a biosystematic study of the genus *Cryptomyzus*

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On Aphids, their Host Plants and Speciation

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J. Adriaan Guldemond

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stellingen

1. "Subspecies" van bladluizen zijn zelden subspecies.
   Müller, F.P. (1986) The role of subspecies in aphids for applied entomology. *Journal of applied Entomology*, **101**, 295-303.

2. Waardwisseling wordt ten onrechte als polyfagie beschouwd.
   Jermy, T. (1984) Evolution of insect/host plant relationships. *American Naturalist*, **124**, 609-630.

3. "Constraint" noch "optimalization" verklaren waardwisseling van bladluizen volledig.
   Moran, N.A. (1988) The evolution of host-plant alternation in aphids: evidence for specialization as a dead end. *American Naturalist*, **132**, 681-706.
   Mackenzie, A. & Dixon, A.F.G. (in press) Host alternation in aphids: constraint versus optimalization. *American Naturalist*.

4. Het proces van soortvorming laat zich ontrafelen door kunstmatige selectie op waardplantgeschiktheid.
   Shaposhnikov, G. Ch. (1966) Origin and breakdown of reproductive isolation and the criterion of the species. *Entomological Review* **45**, 1-18.

5. Fylogenetische analyse van allozymdata met "Jelly" kan onvoorstelbare kenmerktoestanden in de hypothetische vooroudersoorten opleveren.
   Ellis, W.N. (1987) Jelly, version 1.06; a program for the Macintosh computer for the generation of Wagner character state networks using allele frequency characters. Available free of charge from the author.

6. De verspreidingskaarten uit de "Atlas van de Nederlandse Vogels" zeggen vaak meer over verspreiding van vogelaars dan van vogels.
   SOVON, 1987

7. Samenwerking tussen boeren en vogelaars is de enige manier waarop wij de weidevogelpopulaties kunnen behouden.

8. Het vertalen van literatuur wordt meer als roeping dan als beroep gezien.

9. De minimale aaibaarheidsfactor van een bladluis vergemakkelijkt haar bestrijding.
   Kousbroek, R. (1969) *De Aaibaarheidsfactor*, gevolgd door *Die Wacht am IJskast*. Negende, geheel herziene druk, 1983. De Harmonie, Amsterdam.

10. Leptosomen zijn geen subspecies.

Stellingen behorend bij het proefschrift "On Aphids, their Host Plants and Speciation: a biosystematic study of the genus *Cryptomyzus*" door J.A. Guldemond.

Wageningen, 31 januari 1990

Contents

Voorwoord

Een voorwoord bij een proefschrift heeft over het algemeen het karakter van een uitbundig en uitgelaten dankbetoon aan allen die op de een of andere manier aan de totstandkoming van het werk hebben bijgedragen. Dit is mogelijk de reden dat het voorwoord, naast de stellingen, tot het best gelezen deel van een proefschrift behoort. Helaas wordt het feestelijk karakter in dit geval overschaduwd door het verlies van twee voor het project zeer belangrijke personen.

Reeds vrij kort na de start van het onderzoek overleed D. Hille Ris Lambers. Als internationaal vermaard bladluisspecialist was hij mijn buitenuniversitaire begeleider en co-promotor. Weinig mensen zullen zijn uitgebreide ervaring met bladluizen evenaren. Hij was een bijzonder en veelzijdig persoon. Een zo mogelijk nog zwaardere slag betekende het tragische overlijden van René Cobben, mijn oorspronkelijke promotor en initiatiefnemer van het onderzoek. Ik ben hem dankbaar voor de vrijheid die hij mij gaf het onderzoek naar eigen inzichten vorm te geven. Vaak heb ik met hem en zijn vrouw Wies thuis gelunched om me daarna in de meest *Cryptomyzus*-rijke tuin van Nederland terug te trekken. Het gemis van zijn stimulerende belangstelling en innemende vriendschap ervaar ik nog steeds als een groot persoonlijk verlies.

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Ook op andere fronten vond er ondersteuning plaats. De administratie waar Ans

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Zonder Ebbe Eggers-Schumacher zou dit onderzoek veel beperkter zijn geweest, aangezien hij me op een aantal onbekende vormen van *Cryptomyzus* heeft gewezen. Daarnaast heeft hij ook de basisprincipes en receptuur van de elektroforese bij de sectie geïntroduceerd. Deze methode en de fylogenetische verwerking van elektroforetische gegevens is verder bediscussieerd met vooral Maarten Scheepmaker, Jaap Bakker en Steph Menken. Hans de Jong heeft nuttige tips gegeven voor het chromosoomonderzoek. Het contact met Roger Blackman, Chris Brough, Victor Eastop, Ole Heie, Aulay Mackenzie en Georgi Shaposhnikov heeft nieuwe gezichtspunten opgeleverd en was heel stimulerend.

Michael Latcham en Ninette de Zylva brachten het Engels en Rinke van Couwelaar het Nederlands van verschillende teksten op een acceptabel niveau. Steven van Couwelaar zorgde voor een omslag met verantwoorde vormgeving. Het Leptosomen Eenheids Front (LEF, Amsterdam-Parijs) stond op de bres voor de noodzakelijke ruggegraat in barre tijden. Tenslotte waren Cor en Rob Guldemond-Langelaan als ouders altijd bereid om een helpende hand te bieden.

Introductie & Samenvatting

Deel I: Leven als een bladluis

Dit is een studie over bladluizen van het geslacht *Cryptomyzus*. Iedereen is wel enigszins bekend met bladluizen: plantenzuigende insekten die in een aantal gevallen landbouwgewassen aantasten, plantenvirussen overbrengen en bladmisvormingen veroorzaken. Uitscheiding van bladluizen (honingdauw) bevordert de groei van schimmels. Dit alles bij elkaar kan leiden tot flinke opbrengstverliezen in de landbouw. Daarnaast veroorzaakt honingdauw ook de kleverigheid op een auto wanneer deze onder een linde of iep staat. Alhoewel bladluizen belangrijke schadeverwekkers in land-, tuin- en bosbouw zijn, is hun levenswijze opvallend weinig bekend, zelfs onder biologen.

Bladluizen verschillen, in een aantal opzichten aanzienlijk, van andere insektegroepen. Voor een beter begrip van de volgende hoofdstukken is een korte beschrijving van hun levenswijze wenselijk.

De talrijkheid en schadelijkheid van een aantal bladluizen wordt grotendeels veroorzaakt door hun enorme vermenigvuldigingscapaciteit. Dit is het gevolg van een aantal eigenschappen die kenmerkend zijn voor bladluizen. Ten eerste zijn bladluizen, in tegenstelling tot de meeste andere insekten, vivipaar. Dit houdt in dat er kleine, larvale bladluisjes worden geboren in plaats van dat er eieren worden gelegd. In de ovariolen van deze larfjes ontwikkelen zich reeds hun nakomelingen. Dit leidt tot het in elkaar schuiven van generaties, waarbij de dochter al begint met reproductie terwijl haar moeder daar nog mee bezig is. Ten tweede zijn bladluizen cyclisch parthenogenetisch. Dit betekent dat ze van voorjaar tot herfst alleen maar vrouwtjes produceren zonder voorafgaande bevruchting. Dit geheel vrouwlijke nageslacht geeft hen een dubbel reproductievermogen vergeleken met vormen die zich sexueel voortplanten. Er worden namelijk geen mannetjes gevormd. Dit kan leiden tot een snelle opbouw van bladluispopulaties. Zo zijn bijvoorbeeld tot 400 miljoen graanbladluizen per hectare waargenomen.

Ten slotte wordt in de herfst de vorming van mannetjes en sexuele vrouwtjes in gang gezet door het korter worden van de dagen, waarna paring en sexuele voortplanting kunnen plaats vinden. De bevruchte vrouwtjes leggen nu eieren die overwinteren.

De genetische structuur van een bladluispopulatie wordt sterk door deze cyclisch parthenogenetische reproductie bepaald. Door natuurlijke selectie blijft er iedere herfst slechts een beperkt aantal genotypen over. Daarna veroorzaakt sexuele voortplanting door recombinatie en segregatie genetische variabiliteit. Opvallend is dat bij een aantal bladluissoorten een volledig parthenogenetische (anholocyclische) vorm voorkomt naast een cyclisch parthenogenetische (holocyclische) vorm. Zelfs bestaan er volledig parthenogenetische soorten.

Veel plantesoorten worden door bladluizen benut. Toch zijn de meeste bladluizen specialisten die uitsluitend op één, of een aantal nauw verwante plantesoorten kunnen leven. Er zijn slechts weinig polyfage bladluizen die op planten uit geheel verschillende families kunnen leven. Dit zijn vaak de schadelijke soorten. Ongeveer tien procent van alle bladluissoorten heeft een speciale band met twee volkomen verschillende plantesoorten ontwikkeld, die zij ieder seizoen afwisselend bewonen. Dit wordt waardplantwisseling genoemd. De levenscyclus van dit soort bladluizen, zoals *Cryptomyzus galeopsidis*, is afgebeeld in Figuur Ia.

Deze bladluis legt haar eieren op besseplanten van het geslacht *Ribes*. Deze plant is de primaire of winterwaard, gewoonlijk een houtige plant. In het voorjaar komt uit een overwinterend eitje de stammoeder, fundatrix genoemd. Zij produceert na één of meerdere generaties gevleugelde vrouwtjes die dan naar hennepnetel, *Galeopsis*, migreren. Dit is de secundaire of zomerwaard, gewoonlijk juist een kruidachtige plant. Hierop ontwikkelen zich ongevleugelde en gevleugelde vrouwtjes, die andere individuen van dezelfde plantesoort kunnen koloniseren. In de herfst verandert de waardplantvoorkeur van de gevleugelde vrouwtjes echter, die nu gynoparen worden genoemd. Zij keren terug naar de winterwaard *Ribes*. Hierop produceren zij sexuele, eierleggende vrouwtjes, de oviparen. Tegelijkertijd ontstaan op de zomerwaard mannetjes die ook naar de winterwaard migreren. Deze paren met de vrouwtjes die hun eieren bij knoppen of in spleten van de bast leggen. Dit is een typische levenscyclus voor veel Aphididae, maar variaties op het thema waardwisseling komen voor bij andere bladluisfamilies.

De meerderheid der bladluissoorten, negentig procent, is evenwel niet-waardwisselend; hun levenscyclus is een simpele versie van die der waardwisselende soorten. Figuur Ib laat de levenscyclus zien van *Cryptomyzus alboapicalis*, die permanent leeft op witte dovenetel, *Lamium album*.

De seizoensgebonden levenscyclus laat zien dat er verschillende eenheden (stammoeder, gynopaar etc.) voorkomen binnen één bladluissoort. Deze eenheden zijn de zogenaamde morfen die alle een verschillende functie hebben en soms aanzienlijk in uiterlijk verschillen. Wanneer het ongevleugelde vrouwtje de prinses van de voortplanting is, dan is de stammoeder de koningin. De laatste is geheel toegerust om haar reproductie te vergroten. Het gevleugelde vrouwtje vertegenwoordigt de mobiliteit: zij migreert tussen primaire en secundaire waard of is op zoek naar een andere zomerwaard. Deze mobiliteit gaat ten koste van een vermindering in reproductiecapaciteit. Het ovipare vrouwtje produceert het minste aantal nakomelingen. Zij en het mannetje zijn de enige sexuele morfen in de cyclus. Deze verschillende morfen zijn een opvallend verschijnsel, omdat zij vanwege ongeslachtelijke voortplanting alle genetisch identiek zijn binnen een kloon.

Deel II: *Cryptomyzus*

Na deze algemene inleiding over bladluizen, wordt het geslacht *Cryptomyzus* nader beschreven. Een aantal soorten leeft op bessen, *Ribes*, en migreert ieder seizoen naar lipbloemige kruiden. Andere vormen blijven op de winterwaard (*Ribes*) en weer andere op de zomerwaard (*Lamium*). Volgens de literatuur komen er acht *Cryptomyzus* soorten voor in Europa: vier daarvan zijn waardwisselend, één is niet-waardwisselend en van drie soorten is de levenscyclus onvolledig bekend. Eveneens komen in Europa twee niet-waardwisselende ondersoorten voor. Van drie andere soorten ligt het verspreidingsgebied in Azie.

Een aantal van deze soorten is nauw verwant en uiterlijk moeilijk of niet van elkaar te onderscheiden, maar zij verschillen in waardplanten en levenscycli. Hieruit zou geconcludeerd kunnen worden dat verschillende vormen zich in een soortvormingsproces bevinden. Dit biedt de mogelijkheid de rol van waardplant- en levenscyclusdifferentiatie in dit proces te bestuderen. Op grond van hun waardplantrelaties en unieke morfologische kenmerken is het aannemelijk dat de verschillende *Cryptomyzus* vormen van een gemeenschappelijke voorouder afstammen (monofyletisch). Dit maakt het mogelijk om ten eerste het patroon van soortvorming te bepalen, dat tot uitdrukking komt in de afstammingsgeschiedenis (fylogenie). Vervolgens kan het proces van soortvorming, name-

lijk de overgang naar andere waardplanten en levenscycli, worden bestudeerd. Deze eigenschappen maken de soorten en vormen van het *Cryptomyzus*-complex bijzonder geschikt voor een biosystematische studie.

Tijdens het onderzoek bleek dat uit biosystematisch oogpunt de meest belovende groep bestond uit *C. galeopsidis* en *C. alboapicalis*. Deze twee soorten bestaan uit verschillende vormen, die gekenmerkt worden door hun waardplanten en levenscycli (Figuur op uitvouwpagina aan het einde van het proefschrift). Een groot deel van dit onderzoek is gericht op deze vormen en beoogt:

* het ontrafelen van de taxonomie, levenscycli en waardplantrelaties van *Cryptomyzus*,
* het vaststellen van biochemische differentiatie tussen de Europese soorten en op basis hiervan hun fylogenetische relaties,
* het nagaan van de invloed van waardplantvoorkeur en waardplantgeschiktheid op reproductieve isolatie tussen nauw verwante vormen,
* het bepalen of hybridisatie mogelijk is tussen nauw verwante vormen die enkel verschillen in waardplant en levenscyclus,
* het zoeken naar morfometrische verschillen tussen nauw verwante vormen die kunnen wijzen op hun differentiatie,
* het analyseren van de invloed van waardwisseling en waardplantdifferentiatie op het proces van soortvorming.

Deel III: Samenvatting

Allozym gegevens, verkregen door middel van zetmeel-electroforese, tonen aan dat alle *Cryptomyzus* soorten onderscheiden kunnen worden op basis van unieke allelen. Bovendien werden er verschillen gevonden tussen twee vormen van *C. alboapicalis*, die niet-waardwisselend leven op respectievelijk witte dovenetel (*Lamium album)* en gevlekte dovenetel (*L. maculatum*). Ook in het *C. galeopsidis* complex werd een vorm onderscheiden, die waardwisselt tussen rode bes (*Ribes rubrum*) en gele dovenetel (*L. galeobdolon*). De twee niet-waardwisselende ondersoorten op respectievelijk rode en zwarte bes (*R. nigrum*) verschilden in allozymfrequentie, wat duidt op een verminderde uitwisseling van

genetisch materiaal (gene flow). Electroforese bleek een uitstekende methode om de relaties tussen nauw verwante bladluiscomplexen te ontrafelen (Hoofdstuk 1).

De levenscyclus van *C. heinzei* werd opgehelderd door de ontdekking van de winterwaard, alpenbes (*Ribes alpinum*), en de zomerwaard, betonie (*Stachys officinalis*). Op de oorspronkelijk beschreven zomerwaard van deze soort, borstelkrans (*Satureja vulgaris*), bleken in laboratoriumexperimenten geen populaties te kunnen overleven. Dit betekent waarschijnlijk dat de plant verkeerd werd geïdentificeerd. Uit experimenten bleek de winterwaard van *C. ballotae* de alpenbes te zijn, hoewel de reproductie hier matig en de ontwikkeling langzaam waren. Verder wordt een volledig overzicht van de waardplanten van *Cryptomyzus* gegeven (Hoofstuk 2).

Nauw verwante vormen van *C. alboapicalis* en *C. galeopsidis* vertonen een duidelijke preferentie voor hun eigen zomerwaard. Dit was in overeenstemming met de waardplantgeschiktheid, die gebaseerd is op het reproductiesucces (reproductive performance) van deze vormen. Voor het ontstaan van reproductieve isolatie is het van belang dat de morfen die naar de primaire waard terugkeren, kiezen voor de waardplant waar hun stammoeder is geboren. Uit experimenten bleek dit het geval bij waardwisselende klonen van *C. galeopsidis* afkomstig van zwarte bes. De voorkeur van klonen afkomstig van rode bes was niet eenduidig. Verschillende van deze klonen kozen voor rode bes, waarop ook hun oviparen opgroeiden, maar andere hadden een voorkeur voor zwarte bes, waarop hun oviparen eveneens tot volledige ontwikkeling kwamen. Dit geeft aan dat er genetische uitwisseling tussen deze vormen plaats vindt. De populaties met dit intermediaire gedrag zijn mogelijk hybriden. De vormen op rode en zwarte bes worden als waardplantrassen (host races) beschouwd (Hoofdstuk 3).

Kruisingsexperimenten werden uitgevoerd met nauw verwante vormen van *C. galeopsidis*, die samen voorkomen op de winterwaard rode bes. Zij bezitten daarintegen verschillende zomerwaardplanten, namelijk hennepnetel en gele dovenetel. Er werd een eerste kruisingsgeneratie (F_1) verkregen, maar het reproductiesucces van deze kruising was op beide secundaire waardplanten lager dan die van de ouders. Zowel de F_2 als een terugkruising vertoonden een zwakke reproductie op de winterwaard en deze populaties

stierven dan ook uit. Dit maakt het aannemelijk dat een eventuele hybridisatie van deze vormen in het veld waarschijnlijk niet zal resulteren in permanente populaties. Deze hybriden zullen door natuurlijke selectie verdwijnen.

F_1-hybriden werden ook gebruikt om de genetische basis van reproductiesucces en waardplantpreferentie vast te stellen. Dit doel kon niet volledig worden gerealiseerd vanwege de inferioriteit van de hybriden. De resultaten duiden erop dat waardplantvoorkeur mogelijk door weinig genen wordt bepaald en reproductiesucces door vele.

Hybriden tussen waardwisselende en niet-waardwisselende vormen van *C. galeopsidis* vertoonden geen hybride-inferioriteit en deze kruisingen komen waarschijnlijk ook voor in het veld. De resultaten van deze kruisingen maken het aannemelijk dat de aan- of afwezigheid van waardwisseling door slechts één gen (complex) wordt bepaald. Welke gevolgen dit voor soortvorming kan hebben wordt bediscussieerd (Hoofdstuk 4).

Om vast te stellen of de vormen van *C. alboapicalis* en *C. galeopsidis*, die werden onderscheiden op basis van hun waardplanten en levenscycli, ook morfologisch van elkaar verschillen, werd een morfometrische studie uitgevoerd. Daaruit bleek dat *C. alboapicalis* van witte dovenetel onderscheiden kan worden van de andere vormen door het grotere aantal haren op de achterlijfssegmenten. Een analyse met behulp van canonische assen (canonical variate analysis) toegepast op ongevleugelde vrouwtjes, liet zien dat ook *C. alboapicalis* van gevlekte dovenetel aanzienlijk van de andere vormen verschilt. Een lineaire discriminant functie, gebaseerd op de vier beste kenmerken, geeft een goede scheiding van deze vorm met de andere vormen. *C. galeopsidis* van gele dovenetel is nauwer verwant met de andere *C. galeopsidis* vormen en de lineaire discriminant functie is hier minder betrouwbaar. De vier waardwisselende en niet-waardwisselende vormen van *C. galeopsidis* zijn nauw verwant en er is geen eenduidige morfometrische ondersteuning voor de scheiding van de vormen op rode en zwarte bes (Hoofdstuk 5).

De taxonomische conclusies die uit deze studie volgen zijn:

* *C. (Ampullosiphon) stachydis* (Heikinheimo) behoort tot *Cryptomyzus* vanwege haar waardplantrelaties (*Ribes* - Labiatae) en de aanwezigheid van een filterkamer in de darm.

* *C. heinzei* Hille Ris Lambers werd terecht als soort beschreven en verschilt van de morfologisch vergelijkbare *C. korschelti* Börner door het gebruik van andere waardplanten en het bezit van unieke allozymen.
* De *C. alboapicalis* vorm die alleen op gevlekte dovenetel leeft is een aparte soort, genaamd *C. ulmeri* (Börner). Deze was eerder gesynonymiseerd met *C. alboapicalis* (Theobald) van witte dovenetel, maar verschilt van deze soort en van *C. galeopsidis* (Kaltenbach) vormen door haar waardplantvoorkeur, reproductiesucces, levenscyclus, allozymen en morfometrie.
* De *C. galeopsidis* vorm die waardwisselt tussen rode bes en gele dovenetel is een aparte soort en wordt geschreven als *C. maudamanti* **sp.n.**. Deze verschilt van *C. galeopsidis* sensu strictu door haar waardplantvoorkeur, reproductiesucces, allozymen en de inferioriteit van hun hybriden.
* Binnen *C. galeopsidis* kunnen de vormen van rode bes onderscheiden worden van die van zwarte bes. Gebaseerd op waardplantvoorkeur, reproductiesucces en allozymfrequenties worden deze vormen als waardplantrassen beschouwd. Er zijn echter geen aanwijzingen voor een taxonomische scheiding tussen waardwisselende en niet-waardwisselende vormen die op dezelfde primaire waardplant voorkomen. Dit maakt het gebruik van subspecifieke namen voor de niet-waardwisselende vormen, *C. galeopsidis citrinus* HRL op rode bes en *C. galeopsidis dickeri* HRL op zwarte bes, ongewenst.
* Een determinatietabel voor ongevleugelde en gevleugelde vrouwtjes van alle Europese *Cryptomyzus* soorten wordt gegeven (Hoofdstuk 5).

Mogelijke soortvormingswegen van *Cryptomyzus* worden bediscussieerd. Hiertoe werd eerst een stamboom (cladogram) voor dit geslacht geconstrueerd, gebaseerd op gegevens van allozymen, levenscyclus en morfologie. Er bleek een nauw verband aanwezig tussen de taxonomische relaties van *Cryptomyzus* en die van haar waardplanten. Dit kan mogelijk ontstaan zijn door een "navolgende" evolutie (sequential evolution; Jermy 1984). Een meer algemene discussie wordt gevoerd over het proces van soortvorming bij bladluizen en wat de rol van een overgang naar een andere waardplant hierbij kan zijn. Aangezien bladluizen cyclisch parthenogenetisch zijn en een nauwe relatie met een specifieke waardplant vertonen, lijken zij goede kandidaten voor een evolutie via het model van sympatrische soortvorming.

Referenties

Dixon, A.F.G. (1985) Aphid ecology. Blackie, Glasgow.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp. Roy. Ent. Soc. London **6**, London.

Hille Ris Lambers, D. (1966) Polymorphism in Aphididae. Ann. Rev. Entomol. **11**, 47-78.

Jermy, T. (1984) Evolution of insect/plant relationships. Am. Nat. **124**, 609-630.

Watt, A.D. (1984) Reproductive strategies of the alate and apterous morphs of the grain aphid, *Sitobion avenae*. Entomol. exp. appl. **36**, 1-7.

Introduction & Summary

Part I: The life of an aphid

This study concerns aphids belonging to the genus *Cryptomyzus*. Everyone is more or less familiar with aphids: plant sucking insects some of which attack agricultural crops, transmit plant viruses and cause leaf deformation, and whose excretion (honeydew) promotes fungal growth, which collectively may cause severe crop losses. Moreover they are also responsible for the stickiness on a car, if parked under a lime or elm tree. Although major pests of agriculture, horticulture and forestry, even among biologists, surprizingly little is known about their way of life. Aphids differ, and in some respects quite considerably, from other groups of insects, therefore in order to effect a better understanding of the following chapters a short introduction to their way of life is provided.

The great abundance and pest status of some aphids is largely determined by their prodigious reproductive potential. This is achieved in several ways typical of aphids. Firstly, in contrast to most other insects, they are viviparous, which means that they give birth to larval aphids instead of eggs. These larvae are born with their offspring already developing inside their ovarioles resulting in the so-called telescoping of generations, in which the daughters start to reproduce before their mothers have finished. Secondly, aphids are cyclically parthenogenetic, implying that from spring until autumn only females are produced without fertilization. The production of all-female offspring gives them a two-fold reproductive advantage over sexually reproducing forms, because no effort is wasted in producing males. This leads to an enormous and rapid production of offspring; up to 400 million cereal aphids per hectare have been recorded. Finally, in autumn the production of males and sexual females is initiated by short-days, after which sexual reproduction occurs and overwintering eggs are layed.

The genetic structure of an aphid population is thus largely determined by parthenogenetic reproduction. As a result of natural selection only a reduced number of genotypes remain each autumn. Subsequently, sexual reproduction generates genetic

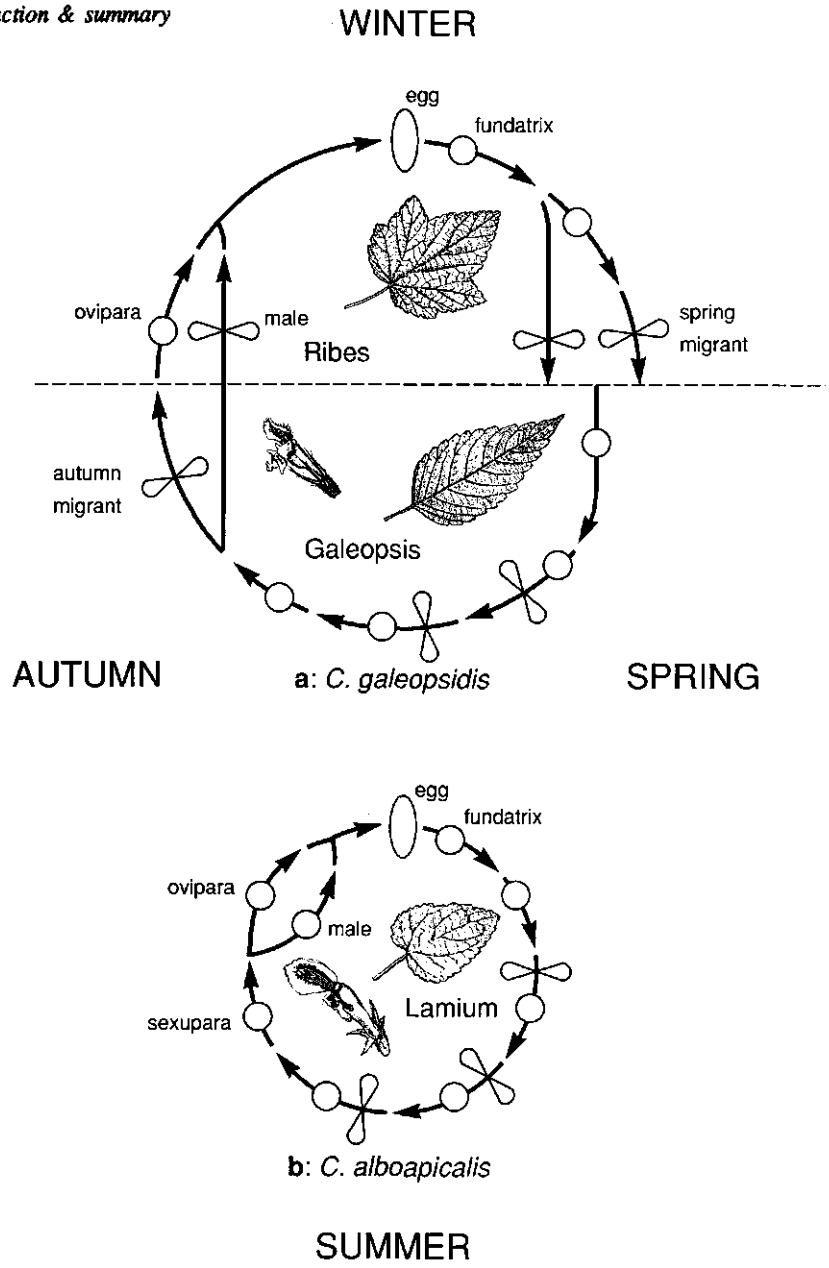

Figure I Life cycle of host-alternating (above) and non-alternating (below) species of *Cryptomyzus*. O are wingless and ⋈ are winged morphs. Arrows do not indicate the exact number of generations (drawing Piet Kostense).

variability by recombination and segregation. Remarkably, in several aphid species completely parthenogenetic (anholocyclic) forms coexist with cyclically parthenogenetic (holocyclic) forms, and some even entirely parthenogenetic.

Many plant species are exploited by aphids. Nevertheless, most aphid species are highly specific and only live on one, or a few closely related plant species. Polyphagous aphids, which can live on plants belonging to different families, are few in species, such aphids constituting often significant pests. About ten percent of all species of aphids have developed a special relationship with two, completely unrelated plant species, between which they migrate seasonally. This is called host-alternation and the life cycle of such aphids is exemplified by that of *Cryptomyzus galeopsidis* (Figure Ia).

This aphid lays its overwintering eggs on the currant, *Ribes*. This is the primary or winter host; usually a woody plant. In spring the stem mother (fundatrix) hatches from an overwintering egg and after one or more generations winged females are produced, which migrate obligatorily to hempnettle, *Galeopsis*. This plant, the secondary or summer host, is usually a herbaceous plant. Here, wingless and winged females develop, which may colonize other plants of the same species. In autumn, however, the host preference of the winged females (gynoparae) is changed, and they return to *Ribes*, the winter host, where they produce sexual, egg-laying females (oviparae). Simultaneously, males develop on the summer host and also return to the primary host. Oviparae and males mate and eggs are layed near buds or in crevices of the bark. This life cycle is typical of many Aphididae, but variations on this theme of host-alternation exist in other aphid families.

The majority of aphids, ninety percent, do not host-alternate and their life cycle is a simplified version of the host-alternating one. Figure Ib shows the life cycle of *Cryptomyzus alboapicalis*, which lives permanently on the white dead-nettle, *Lamium album*.

The seasonal life cycle emphasizes the existence of several distinct units (stem mother, gynopara etc.) occuring in one aphid species. These units are the so-called morphs, all of which have a specialized function and may differ considerably in their morphology. Table I lists the terminology used for these morphs and related terms. If the wingless female is the princess of reproduction then the stem mother is the queen. The latter is entirely devoted to maximizing her reproduction. The winged female represents mobility: alternating between primary and secondary hosts or searching for another summer host, this mobility causes reduction in reproductive capacity. The oviparous

Table I Aphid terminology after Hille Ris Lambers (1966), with common name in *italics*.

alate	*winged*	
apterous	*wingless*	
virginopara		viviparous parthenogenetic female, which produces other viviparous parthenogenetic females
fundatrix	*stem mother*	parthenogenetic female that hatches from a fertilized egg
emigrant	*spring migrant*	virginopara that migrates from primary to secondary host
exule	*summer form* or *summer migrant*	virginopara that originates from and disperses to a secondary host
gynopara	*autumn migrant*	parthenogenetic female that migrates from a secondary to a primary host and produces oviparae
sexupara		parthenogenetic female that produces both oviparae and males, and may also migrate to primary host
	presexual	gynopara and sexupara
ovipara	*egg-laying female*	sexual female that produces eggs after fertilization
primary host	*winter host*	for host-alternating species the plant on which sexual reproduction takes place, eggs are deposited and the fundatrix reproduces
secondary host	*summer host*	for host-alternating species the plant on which only parthenogenetic reproduction takes place
heteroecious/ dioecious	*host-alternating*	seasonal, obligatory migration between two different host plants
autoecious/ monoecious	*non-alternating*	no seasonal, obligatory migration between two different host plants
holocyclic		(life cycle) with alternation of asexual and sexual reproduction
anholocyclic		(life cycle) with only asexual reproduction

female is, together with the male, the only sexual morph in the cycle, and she displays the lowest fecundity. The various morphs are a remarkable phenomenon of aphids, because they are genetically identical since we are dealing with parthenogenetic propagation.

Part II: *Cryptomyzus*

Following this general introduction about aphids, the genus *Cryptomyzus* will be dealt with in greater detail. Several species live on the currants, *Ribes*, and migrate seasonally to labiateous herbs. Others remain either on the winter host (*Ribes*) or on the summer host (*Lamium*). According to the literature, there are eight species of *Cryptomyzus* in Europe: four host-alternating, one non-alternating species, and three species with incompletely known life cycles. Two non-alternating subspecies are also included (Table 1.1).

Three other species occur in Asia.

Several of these species are closely related and are morphologically difficult or impossible to distinguish, but differ in their host plant relationships and life cycles. Accordingly it may be concluded that several forms are in the process of speciation, hence presenting an opportunity to study the role of host plant and life cycle differentiation in speciation. Because of their host plant relationships and unique morphological characters *Cryptomyzus* is likely to have evolved from a common ancestor (mono-phyletic). This makes it possible to determine the pattern of speciation, which finds expression in the phylogeny, and to determine the different switches in host plants and life cycles. These features make the complex of species and forms of *Cryptomyzus* particularly suitable for a biosystematic study.

During the study it became apparent that the most promising biosystematical group constituted *C. galeopsidis* and *C. alboapicalis*. These two species include several forms characterized by their host plants and life cycle (Figure on fold-out page at the end of the thesis). A great deal of this study was focussed on these forms.

The goals of this study are:

* to unravel the taxonomy, life cycles and host plant relationships of *Cryptomyzus*,
* to assess biochemical differentiation between the European species and deduce their phylogenetic relationships,
* to determine the influence of host plant preference and reproductive performance on reproductive isolation of closely related forms,
* to ascertain whether hybridisation is possible between closely related forms, only differing in host plants and life cycle,
* to examine morphometric variation between closely related forms, which may display their differentiation,
* to analyse the influence of host-alternation and host plant differentiation on the process of speciation.

Part III: Summary

Allozyme data as determined by starch gel electrophoresis revealed that all species of *Cryptomyzus* could be distinguished on the basis of unique alleles. Moreover, differences

were detected between the two forms of *C. alboapicalis*, which do not host-alternate and instead live on *Lamium album* and *L. maculatum*, respectively. In the *C. galeopsidis* complex as well a form that host-alternates between *Ribes rubrum* and *L. galeobdolon* was distinguished. The two non-alternating subspecies on *R. rubrum* and *R.nigrum*, respectively, differed in allozyme frequency, indicating a reduced gene flow. Electrophoresis proved a powerful tool in unraveling relationships of closely related aphid complexes (Chapter 1).

The life cycle of *C. heinzei* was elucidated by the discovery that *Ribes alpinum* is the winter host and *Stachys officinalis* the summer host. No populations survived on the originally described summer host plant of this species, *Satureja vulgaris*, probably indicating that the host plant was initially misidentified. In laboratory experiments *R. alpinum* appeared to be the winter host of *C. ballotae* although reproduction and development on this plant were weak. A full account of the host plants of *Cryptomyzus* species is given in Chapter 2.

Closely related forms of *C. alboapicalis* and *C. galeopsidis* revealed a definite host plant preference for their own particular summer host plant. This fact was corroborated by the host plant suitability, based on the reproductive performance of these forms. Significantly, for the development of reproductive isolation those morphs returning to the winter host exhibit a prefererence for the host on which their stem mother was born. Experiments confirmed this feature in the case of host-alternating clones of *C. galeopsidis* from *R. nigrum*, although host preference of those from *R. rubrum* proved to be ambiguous. Several clones preferred *R. rubrum* on which their oviparae matured, while others preferred *R. nigrum* on which their oviparae matured as well. This indicates that gene flow occurs between these forms. The populations with intermediate behaviour may be assumed to be hybrids and the forms on *R. rubrum* and *R. nigrum* are considered to represent host races (Chapter 3).

Hybridization experiments were performed between the closely related forms of *C. galeopsidis*, which share the winter host *R. rubrum*, but have different summer hosts, *Galeopsis* and *Lamium galeobdolon*, respectively. A F_1 generation could be established, but its fecundity on both summer hosts was lower than that of the parents. The F_2 and a

backcross revealed that reproduction on the winter host was weak and the populations subsequently died out. This demonstrates that hybridisation of these two forms in the field would probably not result in permanent populations and natural selection would eliminate these hybrids.

F_1 hybrids were also used to determine the genetic basis of reproductive performance and host preference. This objective could not be fully realized, because of hybrid inferiority. Preliminary results indicate that preference may be determined by only a few genes and reproductive performance by many.

Hybrids between the host-alternating and non-alternating forms of *C. galeopsidis* revealed no hybrid inferiority, and probably hybridization also occurs in the field. The results of these crosses argue for a one gene (complex) determination of host-alternation. The implications of this for speciation are discussed (Chapter 4).

A morphometric study was initiated to determine whether the forms of *C. alboapicalis* and *C. galeopsidis*, described on the basis of their hosts and life cycles, are also morphologically distinct. *C. alboapicalis* from *L. album* can be differentiated by the greater number of hairs on their abdominal segments. A canonical variate analysis using wingless females showed that *C. alboapicalis* on *L. maculatum* deviates considerably in morphology from the other taxa. A linear discriminant function, which uses the best four characters, adequately separates this taxon. *C. galeopsidis* on *L. galeobdolon* is more closely related to the other *C. galeopsidis* forms, and the linear discriminant function is less reliable. The four host-alternating and non-alternating forms of *C. galeopsidis* are closely related, and there is no unequivocal morphometric support for the separation of the forms on *R. rubrum* and *R. nigrum* (Chapter 5).

The taxonomic conclusions that emerge from this study are:

* *C. (Ampullosiphon) stachydis* (Heikinheimo) belongs to *Cryptomyzus* on the basis of its host plant relationships (*Ribes* and Labiatae) and the presence of a filter-chamber in its gut.
* *C. heinzei* Hille Ris Lambers is a separate species which differs from the morphologically comparable *C. korschelti* Börner in having different host plants and unique allozymes.
* The form of *C. alboapicalis* that only lives on *L. maculatum* is a separate species

with the name *C. ulmeri* (Börner). It was previously synonymized with *C. alboapicalis* (Theobald) that lives on *L. album*, but it differs from this species and *C. galeopsidis* (Kaltenbach) forms in its host plant preference, reproductive performance, life cycle, allozymes and morphometrics.

* The form of *C. galeopsidis* that host-alternates between *R. rubrum* and *L. galeobdolon* is a separate species and described as *C. maudamanti* **sp.n.**. Its host plant preference, reproductive performance, allozymes and the inferiority of its hybrids conclusively show that it differs from *C. galeopsidis* sensu strictu.
* Within *C. galeopsidis* the forms on *R. rubrum* can be distinguished from those of *R. nigrum*. On the basis of host preference, reproductive performance and allozyme frequency these forms are called host races. Contrastly, there is no support for taxonomically separating the host-alternating and non-alternating forms living on the same primary host plant. This makes the use of subspecific names for the non-alternating forms, *C. g. citrinus* HRL on *R. rubrum* and *C. g. dickeri* HRL on *R. nigrum*, undesirable.
* A key for wingless and winged virginoparous females was constructed for all European species of *Cryptomyzus* (Chapter 5).

The possible pathways of speciation in *Cryptomyzus* are discussed. Therefore, a phylogeny was presented for this genus, based on allozyme, life cycle and morphological characters. A close association between the taxonomic relationships of *Cryptomyzus* and its host plants appeared, which was suggested to have been originated by sequential evolution (Jermy 1984). The process of speciation is discussed more generally, and the role of a host plant shift is considered. Because aphids have cyclical parthenogenesis and a close association with a specific host plant, they seem good candidates to follow the mode of sympatric speciation (Chapter 6).

References

Dixon, A.F.G. (1985) Aphid ecology. Blackie, Glasgow.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp.

Roy. Ent. Soc. London **6**, London.

Hille Ris Lambers, D. (1966) Polymorphism in Aphididae. Ann. Rev. Entomol. **11**, 47-78.

Jermy, T. (1984) Evolution of insect/plant relationships. Am. Nat. **124**, 609-630.

Watt, A.D. (1984) Reproductive strategies of the alate and apterous morphs of the grain aphid, *Sitobion avenae*. Entomol. exp. appl. **36**, 1-7.

1 Biosystematics of the aphid genus *Cryptomyzus*: an electrophoretic analysis [1]

ABSTRACT

The aphid genus *Cryptomyzus* was studied using starch gel electrophoresis in order to establish differences between the various taxa and to estimate their phylogenetic relationships. A low degree of polymorphism and heterozygosity was observed. Taxa previously assumed to be homogeneous appeared to consist of different host-specific forms. Polymorphism at the PGI locus was used to assess the degree of isolation. It was found to range from complete separation to a reduction in gene flow. Three methods of estimating phylogenetic relationships were employed: the UPGMA clustering method using Nei's genetic distance; the Rogers distance together with the distance Wagner method and the independent allele model of Mickevich & Mitter (1981) combined with the Wagner parsimony method. The results of all three methods agree that several of the taxa are closely related but assign different lower branching points to the phylogenetic tree. The independent allele model is discussed in more detail because it is not often applied.

key words: *Cryptomyzus*, aphids, Aphididae, allozymes, electrophoresis, phylogenetic analysis, polymorphism, gene flow.

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[1] with H.A. Eggers-Schumacher. Zeitschrift für zoologische Systematik und Evolutionforschung 27 (1989), 14-25.

INTRODUCTION

An extensive and excellent description of the morphology and biology of *Cryptomyzus* Oestlund (Homoptera: Aphididae) is provided by Hille Ris Lambers (1953). Eastop & Hille Ris Lambers (1976) recognize eight European species and two subspecies. Four species host-alternate between primary host plant species of the genus *Ribes* and secondary host plant species of the family Labiatae.

One species is monoecious on *Lamium album* and the two subspecies are non host-alternating and live on *Ribes rubrum* and *R. nigrum*, respectively. The life cycle of three species is unknown (Table 1.1). The taxonomic status of one of these species, *C. heinzei*, and of both subspecies, is uncertain (Hille Ris Lambers 1953). Three other species have been described from Asia (Eastop & Hille Ris Lambers 1976; Narzikulov & Daniarova 1979). The species *C. ribis* forms red blisters on *Ribes* and can be noxious to commercial currant production due to virus transmission and contamination of the foliage with honeydew, which often supports fungal growth.

Earlier allozyme studies on aphids showed low levels or an absence of intraspecific variation (May & Holbrook 1978; Wool et al. 1978; Furk 1979; Rhomberg et al. 1985; Brookes & Loxdale 1987). Studies on the population genetics of aphids have demonstrated variation in electromorphs correlated with population density (Tomiuk & Wöhrmann 1981), season (Rhomberg et al. 1985) and geographic distribution (Tomiuk & Wöhrmann 1984; Loxdale et al. 1985; Steiner et al. 1985). Little of the electrophoretic variation within a species seems to be correlated with host plant species (Furk 1979; Simon et al. 1982). In contrast, other characteristics e.g. colour and reproduction, were found to correlate with host plant species (Weber 1985; Takada 1986).

In taxonomic studies of aphids, electrophoretic differences have been demonstrated between species of the same genus and species of different genera (Tomiuk et al. 1979; Odermatt 1981; Loxdale et al. 1983; Tomiuk & Wöhrmann 1983). In one study a form previously not described was discovered electrophoretically (Singh & Rhomberg 1984). Electrophoretic data have also been used to establish phylogenetic relationships (Tomiuk & Wöhrmann 1983; Eggers-Schumacher 1987).

The present study investigates whether there are electrophoretic grounds for recognizing the *Cryptomyzus* taxa. In this connection the association of differences in

life cycle or host plant relation and electrophoretic variation within a taxon was considered. Finally, the electrophoretic data were used to estimate the phylogenetic relationships of the *Cryptomyzus* taxa.

MATERIALS AND METHODS

Material

All European species of *Cryptomyzus* were studied, except *C. leonuri* Bozhko. Table 1.1 lists the taxa and their host plant relationships.

Because aphids are cyclically parthenogenetic, there is a risk of sampling only a few genotypes of each taxon. Therefore, many different localities were visited and up to 25 specimens per locality were collected.

Table 1.1 The European species of *Cryptomyzus* according to Eastop & Hille Ris Lambers (1976), their abbreviations used in the figures, the host plants and the number of sampling localities. In parentheses the number of localities from outside the Netherlands. The samples of *C. alboapicalis* and *C. galeopsidis* are divided in origin from host plant e.g. *C. alboapicalis* collected from *L. maculatum* etc.

taxon	abbreviation	primary host plant	secondary host plant	number of sampling localities
C. leonuri Bozhko	*leonuri*	?	*Leonurus cardiaca*	0
C. heinzei Hille Ris Lambers	*heinzei*	?	*Stachys officinalis*	3(3)
C. ballotae Hille Ris Lambers	*ballotae*	?	*Ballota nigra*	6(1)
C. ribis (Linné)	*ribis*	*R. rubrum/ nigrum*	*Stachys* spp.	37(4)
C. korschelti Börner	*korsch*	*R. alpinum*	*Stachys sylvatica*	16(4)
C. alboapicalis (Theobald)				
from *L. maculatum*	*albo*(Lm)	-	*Lamium maculatum*	10(3)
from *L. album*	*albo*(La)	-	*Lamium album*	17(6)
C. galeopsidis (Kaltenbach)				
from *Galeopsis* spp.	*gal*(Gt)	*R. rubrum/ nigrum*	*Galeopsis* spp.	68(8)*
from *L. galeobdolon*	*gal*(Lg)	*R. rubrum*	*Lamium galeobdolon*	12(3)
C. g. citrinus Hille Ris Lambers	*citrinus*	*R. rubrum*	-	
C. g. dickeri Hille Ris Lambers	*dickeri*	*R. nigrum*	-	
C. (Ampullosiphon) stachydis (Heikinheimo)	*stach*	*R. rubrum*	*Stachys/Galeopsis*	1(1)

? = host plant is unknown, - = non existing; * = the combined number of the samples of *C. galeopsidis* from *Galeopsis* spp. and of those of the two subspecies.

Over the period 1982-1987 145 samples of *Cryptomyzus* were collected in the Netherlands, mainly from the central part. In addition, a number of taxa were collected from France (2 samples), Great Britain (1), Czechoslovakia (3), Belgium (2), Sweden (3), Finland (1) and West-Germany (20). The number of samples of each taxon is given in Table 1.1. Two species are not present in the fauna of the Netherlands. The *C. stachydis* material consisted of one Finnish clone and *C. heinzei* was only found in West-Germany. A list of sampling localities is available on request (Appendix).

Electrophoresis

Horizontal starch gel electrophoresis was carried out on single individuals, homogenized in gel buffer. A 10% gel (containing 54 % Sigma starch and 46% Electro starch) was used, with a tris-citrate (TC) gel buffer (0.036/0.012 M, pH 7.1, electrode buffer: 3.75 times as concentrated as the gel buffer) or a tris-borate (TB) gel buffer solution (0.087/0.010 M, pH 9.0, gel buffer as concentrated as the electrode buffer). Electrophoresis was performed at 4° C, for 5 hours at 300 V (TB gels) and at 120 V (TC gels).

Staining procedures were those of Shaw & Prasad (1970) and Tomiuk & Wöhrmann (1983) and a 0.125 M tris-HCl staining buffer of pH 7.5 or 8.0 was used. If necessary, samples could be stored at -70° C prior to electrophoresis.

The 12 enzymes examined, yielding 15 presumed loci, were: glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49, on TB gel); sorbitol dehydrogenase (SDH, EC 1.1.1.14, on TB gel); hexokinase (HK-1 and HK-2, EC 2.7.1.1, on TB or TC gel); phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.43, on TC gel); aldolase (ALD, EC 4.1.2.13, on TC gel); adenylate kinase (AK, EC 2.7.4.3, on TC gel); malate dehydrogenase (MDH-1 and MDH-2, EC 1.1.1.37, on TC gel); isocitrate dehydrogenase (IDH-1 and IDH-2, EC 1.1.1.42, on TC gel); phosphoglucoisomerase (PGI, EC 5.3.1.9, on TB or TC gel); malic enzyme (ME, EC 1.1.1.40, on TC gel); glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8, on TC gel) and phosphoglucomutase (PGM, EC 5.4.2.2, on TC gel).

Mobilities of allozymes were assessed relative to those of a clone of the pea aphid, *Acyrthosiphon pisum* (Harris). For HK-2 a clone of the aphid *Aphis fabae* Scopoli was used as reference. Small differences between taxa in enzyme mobility were confirmed by comparing the mobilities side by side on a single gel. Allozymes were

lettered in order of anodal migration.

Phylogeny

Three methods of dendrogram construction were used. First, Nei's (1972) genetic distance measure between pairs of taxa was subjected to the UPGMA clustering technique (Sneath & Sokal 1973). Secondly, the distance measure of Rogers (1972) was combined with the distance Wagner method of Farris (1972) (Avise 1983). Thirdly, the independent allele model of Mickevich & Mitter (1981) was applied in which the electromorphs of the taxa were coded as either present (1) or absent (0). The resultant matrix was analysed by the Wagner parsimony method using the PHYLIP computer program of J. Felsenstein (version 3.0, PENNY option, all possible trees are calculated).

The most closely related genus to *Cryptomyzus* on the basis of morphology, chromosome number and host plant relationships, was found to be *Nasonovia* (outgroup). Phylogenetic trees were rooted using *N. compositellae nigra* (Hille Ris Lambers), *N. pilosellae* (Börner) and *N. ribisnigri* (Mosley) consecutively. These are three of the four European *Nasonovia* species (Heie 1979). A group of trees was derived for each of the three outgroup species. These were combined in a consensus tree which weighted each group of trees equally (for details see results). The apomorphic (derived) character states defining clades are indicated on the Wagner parsimony tree (Sites et al. 1984).

RESULTS

Interpretation of the electromorphs

The numbers of individuals examined for each taxon and at each locus are listed in Table 1.2. Three out of 15 presumed enzyme loci appeared to be polymorphic in one or more species. Loci were classified as polymorphic when the frequency of their most common electromorph was < 99% (Ferguson 1980).

PGM and HK-1 appeared to have a monomeric structure (two banded heterozygotes), while PGI and 6-PGDH seemed to have a dimeric structure. PGI in *C. galeopsidis* showed a three allele polymorphism with three banded heterozygotes. This polymorphism did not manifest itself on tris-borate gels.

char. nr	enzyme	*heinzei*	*ballotae*	*ribis*	*korsch*	*albo*(Lm)	*albo*(La)	*gal*(Gt)	*gal*(Lg)	*stach*	*N. ribisn*	*N. nigra*	*N. pilos*
1-2	MDH-1	A=1.00 n=5(3)	A=1.00 n=25(4)	A=1.00 n=80(12)	A=1.00 n=36(6)	A=1.00 n=31(3)	A=1.00 n=60(8)	A=1.00 n=146(17)	A=1.00 n=10(3)	B=1.00	A=1.00	A=1.00	A=1.00
3	MDH-2	A=1.00 n=3(1)	A=1.00 n=35(5)	A=1.00 n=100(15)	A=1.00 n=50(7)	A=1.00 n=39(4)	A=1.00 n=79(10)	A=1.00 n=268(30)	A=1.00 n=10(3)	A=1.00	A=1.00	A=1.00	A=1.00
4-5	IDH-1	A=1.00 n=3(1)	A=1.00 n=21(3)	B=1.00 n=39(10)	B=1.00 n=53(8)	A=1.00 n=41(3)	A=1.00 n=65(10)	A=1.00 n=277(26)	A=1.00 n=37(7)	A=1.00	A=1.00	A=1.00	A=1.00
6-7	IDH-2	B=1.00 n=5(3)	B=1.00 n=35(5)	B=1.00 n=153(21)	B=1.00 n=40(6)	B=1.00 n=51(5)	B=1.00 n=87(11)	B=1.00 n=395(36)	B=1.00 n=43(7)	A=1.00	B=1.00	B=1.00	B=1.00
8-11	6-PGDH	B=1.00 n=5(3)	B=1.00 n=18(2)	C=1.00 n=74(12)	C=1.00 n=21(5)	C=1.00 n=27(8)	A=0.11 C=0.89 n=53(8)	C=1.00 n=211(20)	C=1.00 n=41(7)	C=1.00	D=1.00	D=1.00	D=1.00
12-15	HK-1	C=1.00 n=8(2)	C=1.00 n=37(3)	D=1.00 n=160(25)	D=1.00 n=131(17)	A=1.00 n=84(11)	A=0.005 B=0.995 n=99(14)	A=1.00 n=248(34)	A=1.00 n=70(10)	C=1.00	C=1.00	C=1.00	C=1.00
16-17	HK-2	A:1.00 n=3(1)	A=1.00 n=3(2)	B=1.00 n=12(4)	A=1.00 n=10(3)	A=1.00 n=7(2)	A=1.00 n=9(3)	A=1.00 n=13(3)	A=1.00 n=10(3)	?	A=1.00	A=1.00	A=1.00
18-21	G-6-PDH	C=1.00 n=8(2)	D=1.00 n=36(3)	A=1.00 n=88(12)	B=1.00 n=70(11)	C=1.00 n=45(5)	C=1.00 n=49(6)	C=1.00 n=89(13)	C=1.00 n=21(2)	A=1.00	A=1.00	?	?
22-25	ME	A=1.00 n=5(3)	A=1.00 n=39(6)	A=1.00 n=178(20)	A=1.00 n=90(12)	B=1.0 n=54(6)	B=1.00 n=95(11)	B=1.00 n=511(53)	B=1.00 n=76(10)	D=1.00	D=1.00	D=1.00	C=1.00
26-31	SDH	B=1.00 n=8(2)	B=1.00 n=6(2)	E=1.00 n=72(9)	D=1.00 n=50(6)	A=1.00 n=13(3)	C=1.00 n=30(4)	C=1.00 n=35(7)	C=1.00 n=3(2)	C=1.00	F=1.00	?	?
32-37	PGM	A=1.00 n=5(3)	A=1.00 n=43(6)	A=0.11 B=0.89 n=154(20)	B=1.00 n=86(11)	E=0.987 D=0.013 n=74(7)	E=0.995 D=0.005 n=104(8)	E=0.998 D=0.002 n=410(43)	E=0.990 D=0.010 n=62(9)	F=1.00	C=1.00	C=1.00	C=1.00
38-43	GPDH	A=1.00 n=5(3)	A=1.00 n=61(6)	D=1.00 n=116(24)	E=1.00 n=71(11)	C=1.00 n=36(5)	E=1.00 n=82(13)	C=1.00 n=168(32)	C=1.00 n=19(4)	C=1.00	B=1.00	B=1.00	B=1.00
44-50	PGI	E=1.00 n=5(3)	A=1.00 n=76(6)	C=1.00 n=172(28)	E=1.00 n=63(8)	B=1.00 n=77(9)	B=1.00 n=144(12)	B=0.305 E=0.649 G=0.047 n=651(83)	D=0.959 B=0.035 G=0.006 n=86(8)	E=1.00	F=1.00	B=0.75 E=0.25	C=1.00
51	ALD	A=1.00 n=3(1)	A=1.00 n=33(3)	A=1.00 n=56(10)	A=1.00 n=38(6)	A=1.00 n=10(1)	A=1.00 n=40(5)	A=1.00 n=176(14)	A=1.00 n=46(6)	A=-1.00	A=1.00	A=1.00	A=1.00
52	AK	A=1.00 n=3(1)	A=1.00 n=33(3)	A=1.00 n=49(11)	A=1.00 n=40(7)	A=1.00 n=16(2)	A=1.00 n=41(6)	A=1.00 n=209(16)	A=1.00 n=58(6)	A=-1.00	A=1.00	A=1.00	A=1.00

An inbred PGI heterozygote clone of *C. galeopsidis* gave a simple Mendelian ratio of 1:2:1 in the F1 (7 slow allozymes, 12 heterozygotes, 10 fast allozymes; $X^2_{(2)}=1.45$, $0.50<P<0.10$).

Polymorphism and heterozygosity

Cryptomyzus species showed a range of polymorphism from 0% to 12.5% and a mean heterozygosity per locus of between 0 and 0.031 (Table 1.3). These results are similar to those obtained in other genetic studies of aphids (Loxdale et al. 1985; Rhomberg et al. 1985 for a summary; Loxdale et al. 1986 for different results on polymorphism). The levels of polymorphism in aphids appeared to be low in comparison with other insects (Nevo 1978).

Electrophoretic variation

The results of Table 1.2 show that all species of *Cryptomyzus* can be distinguished electrophoretically. The status of *C. heinzei* as a separate species is confirmed. It differs from *C. ballotae*, its closest relative, at two enzyme loci (Table 1.2). The relation between intraspecific allozyme variation and host plant species was considered further. *C. heinzei*, *C. ballotae* and *C. korschelti* did not show allozymic variation. *C. ribis* appeared to be polymorphic for PGM, and was collected from its primary host plants *Ribes rubrum* (n=116) and *R. nigrum* (n=10) and the secondary host plants *Lamium amplexicaule* (n=9) and *Stachys palustris* (n=16). No correlation was found between PGM electromorphs and these host plants. Surprisingly, only one PGM heterozygote of *C. ribis* was found, which suggests the existence of two rather isolated forms of this species.

Two distinct forms of the monoecious *C. alboapicalis* were detected. Populations from *Lamium album* and *L. maculatum* showed differences in HK-1, SDH and GPDH. Only one clone from *L. album* also exhibited the heterozygous HK-1 electromorph char-

Table 1.2 Proportion of the electromorphs of each enzyme locus for *Cryptomyzus* taxa and the outgroup species *Nasonovia ribisnigri* [n=23 (7 clones)], *N. compositellae nigra* [n=12(3)] and *N. pilosellae* [n=6(1)]. Different electromorphs of a locus are classified A to G and ranked in terms of increasing anodal migration. The character numbers, used in Figure 1.1C, are ranked for each locus in alphabethcal order. The sample size of *C. stachydis* is one clone.

Table 1.3 Percentage of polymorphism (%P) and the mean heterozygosity per locus (H_L) for *Cryptomyzus* species.

taxon	%P	H_L	taxon	%P	H_L
heinzei	0	0	*alboapicalis*(Lm)	6.3	0.002
ballotae	0	0	*alboapicalis*(La)	18.3	0.014
ribis	6.3	0.012	*galeopsidis*(Gt)	6.3	0.031
korschelti	0	0	*galeopsidis*(Lg)	12.5	0.006

acteristic of populations from *L. maculatum* (Table 1.2). The substantial differentiation that has taken place between these taxa, indicates strong genetic isolation and is good evidence to consider them as separate species.

C. galeopsidis appeared to comprise of a number of forms, which differ in their primary or secondary host plants or type of life cycle (Hille Ris Lambers 1953; Guldemond 1987). The form which host-alternates from *Ribes rubrum* and *R. nigrum* to *Galeopsis* spp. is here called *C. galeopsidis*(Gt), while the form migrating from *R. rubrum* to *Lamium galeobdolon* is called *C. galeopsidis*(Lg). Non host-alternating forms have been described from *R. rubrum*, (*C. g. citrinus*) and from *R. nigrum*, (*C. g. dickeri*). Aphids collected from *L. galeobdolon* and *Galeopsis* spp. only differed in PGI. *C. galeopsidis*(Lg) shows the unique allele D at a frequency of 0.96 (Table 1.2), whereas its other alleles were similar to those of *C. galeopsidis*(Gt). This indicates that gene flow

Table 1.4 Allele frequencies for PGI alleles in *C. galeopsidis* samples from secondary host plants and from the primary host plants *Ribes rubrum* and *R. nigrum*.
Samples from *Ribes* are divided in the putative non host-alternating form, collected in July-September and in a mixture of host-alternating and non-alternating forms from April-June.

samples from		alleles			
hostplant	period	B	E	G	n
Ribes rubrum	July - September	0.355	0.532	0.114	282
Ribes nigrum		0.228	0.763	0.009	114
Ribes rubrum	April - June	0.161	0.732	0.107	56
Ribes nigrum		0.312	0.685	0.004	276
secondary host plants		0.258	0.726	0.016	550

n = pooled sample size of alleles

between the two forms is limited. In the other *C. galeopsidis* forms, no unique electromorphs were found for the enzymes studied.

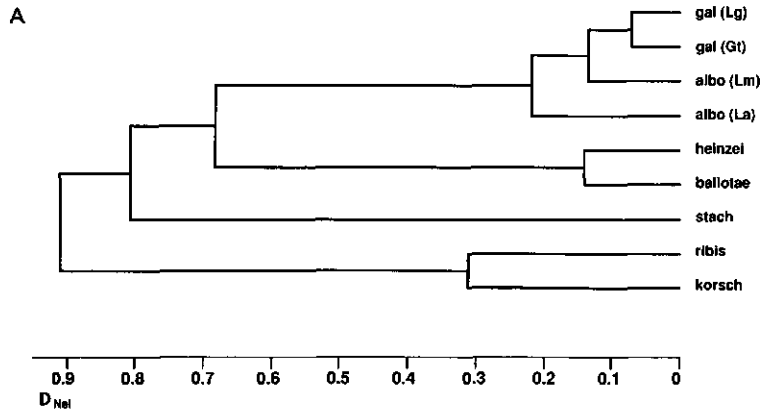
In order to compare the two non host-alternating forms, the July-September samples from both *Ribes* species were used, because these can be assumed to consist of host- alternating forms only (Hille Ris Lambers 1953). The subspecies on *R. rubrum* and *R. nigrum* differed in frequency of PGI (Table 1.4, $X^2_{(3)}$=22.06, P<0.001), which indicates that there is only a limited gene flow between them.

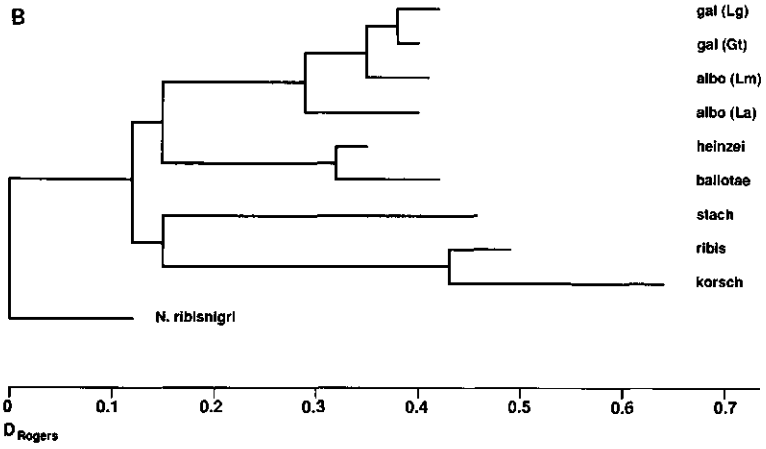
It would be interesting to know if the host-alternating and non host-alternating forms of *C. galeopsidis* can be distinguished electrophoretically. Samples of the host-alternating forms collected from *Galeopsis* spp. and derived either from *R. rubrum* or *R. nigrum*, could not be separated morphologically. Thus, it was impossible to determine how the two forms contributed to the frequency of the three PGI alleles (Table 1.4). It was thought that comparison of the July-September samples with those of April-June from the same *Ribes* species could be informative because the early samples also include some host-alternating forms. Although the *R. nigrum* samples showed no difference when tested ($X^2_{(3)}$=2.72, 0.10<P<0.50), those of *R. rubrum* showed a significant difference ($X^2_{(3)}$=8.85, P<0.05). This might indicate a divergence between the host-alternating and non host-alternating forms from *R. rubrum*.

Phylogeny

It appears that in all methods used here the taxa were clustered in three main groups, *ribis-korschelti*, *ballotae-heinzei* and *galeopsidis-alboapicalis* (Figures 1.1A, B and C, based on Tables 1.2 and 1.5). However, the methods differed in the way in which they relate these groups to each other and how they place *stachydis*.

The Wagner parsimony method used in combination with each of the *Nasonovia* out-group species gave several phylogenetic trees. Electrophoretically, the *Nasonovia* species are closely related to each other and differ only in one or two enzyme loci out of the 13 measured (Table 1.2). Using *N. compositellae nigra* yielded 2 trees of 57 steps; using *N. pilosellae* 10 trees of 58 steps and *N. ribisnigri* 4 trees of 59 steps. These trees differ from Figure 1.1C in the position of the two *alboapicalis* taxa and the relationships between *stachydis* and *ribis-korschelti* to the *galeopsidis-alboapicalis* group.


A
gal (Lg)
gal (Gt)
albo (Lm)
albo (La)
heinzei
ballotae
stach
ribis
korsch
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
D Nei


B
gal (Lg)
gal (Gt)
albo (Lm)
albo (La)
heinzei
ballotae
stach
ribis
korsch
N. ribisnigri
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
D Rogers

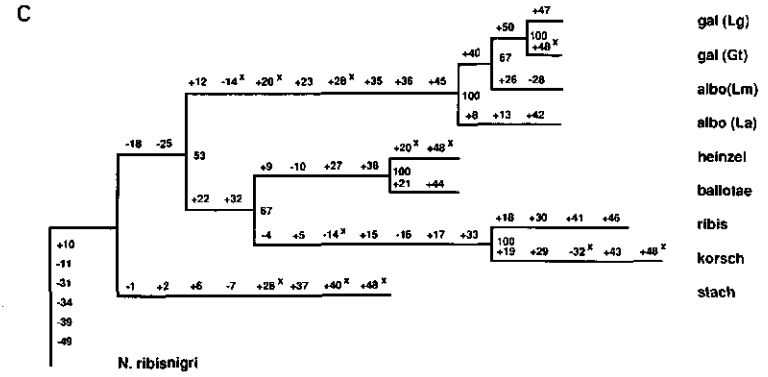

C
gal (Lg)
gal (Gt)
albo(Lm)
albo (La)
heinzei
ballotae
ribis
korsch
stach
N. ribisnigri

Table 1.5 Matrix of Nei's distance measure (below diagonal) and Rogers distance measure (above diagonal, in *italics*) between pairs of taxa of *Cryptomyzus* and an outgroup species *Nasonovia ribisnigri*.

	species	1	2	3	4	5	6	7	8	9	10
1.	*N. ribisnigri*		*0.47*	*0.47*	*0.60*	*0.67*	*0.53*	*0.53*	*0.52*	*0.53*	*0.57*
2.	*heinzei*	0.63		*0.13*	*0.59*	*0.53*	*0.47*	*0.46*	*0.42*	*0.47*	*0.57*
3.	*ballotae*	0.63	0.14		*0.59*	*0.60*	*0.53*	*0.53*	*0.52*	*0.53*	*0.64*
4.	*ribis*	0.92	0.90	0.90		*0.27*	*0.60*	*0.64*	*0.59*	*0.60*	*0.64*
5.	*korschelti*	1.10	0.76	0.92	0.31		*0.60*	*0.64*	*0.56*	*0.60*	*0.64*
6.	*alboapicalis*(Lm)	0.76	0.63	0.76	0.92	0.92		*0.24*	*0.11*	*0.13*	*0.57*
7.	*alboapicalis*(La)	0.76	0.63	0.76	0.92	0.92	0.22		*0.22*	*0.24*	*0.62*
8.	*galeopsidis*(Gt)	0.76	0.52	0.76	0.92	0.78	0.13	0.21		*0.06*	*0.45*
9.	*galeopsidis*(Lg)	0.76	0.63	0.76	0.92	0.92	0.14	0.22	0.07		*0.50*
10.	*stachydis*	0.85	0.85	1.03	1.03	1.03	0.85	0.85	0.57	0.69	

A strict consensus tree, which only gives branching points if these occur in all the trees, showed an unresolved four branched fork with *ribis-korschelti*, *ballotae-heinzei*, *galeopsidis-alboapicalis* andy *stachydis* as terminal taxa. The differences between the various trees probably is due to homoplaseous characters.

Figure 1.1C shows the majority rule consensus tree, which only gives branching points if these occur in more then half of the trees. The calculation of the consensus tree was based on the three groups of trees derived from the three *Nasonovia* outgroup species. For each group the frequency of the different branching points (forks) was calculated. Finally, the mean frequencies for the forks of the consensus tree were computed

Figure 1.1 Three dendrograms of *Cryptomyzus* based on electrophoretic data. Abbreviations of the taxa see Table 1.1. A: UPGMA clustered dendrogram derived from the Nei's distances in Table 1.5. F=8.04% (Prager & Wilson 1976). B: Distance Wagner tree derived from Rogers distances of Table 1.5. *Nasonovia ribisnigri* is used as the outgroup species. F=10.50% (Prager & Wilson 1976). C: Wagner parsimony majority rule consensus tree derived from binary coded characters (52) in Table 1.2. See text for explanation about the calculation of the tree and the numbers at the forks. Characters defining clades are indicated along the branches if *N. ribisnigri* is used as the outgroup species. Character numbers following Table 1.2. + = character has state 1; - = character has state 0; * = homoplaseous characters. Tree with 12 homoplaseous steps.

and these are given as percentages at the base of the forks. For example: with *N. compositellae nigra* as outgroup species, the fork ending with *ribis-korschelti* and *ballotae-heinzei* occurred in 100% of the cases and in 50% of the cases when the other two *Nasonovia* species were used. This gave a mean of (100+50+50)/3=67%.

DISCUSSION

Biosystematics

Electrophoresis proved to be a powerful tool for discriminating between closely related taxa in the genus *Cryptomyzus*. The study revealed that the *C. galeopsidis* complex includes several, biochemically definable forms which show different degrees of reproductive isolation. *C. galeopsidis*(Lg), host-alternating between *Ribes rubrum* and *Lamium galeobdolon*, exhibits the strongest isolation from the other *C. galeopsidis* forms. The non host-alternating taxa on *R. rubrum* and *R. nigrum*, which show only differences in allele frequencies, are more closely related.

The genetic differences found between the non host-alternating and host-alternating forms collected from *R. rubrum* could possibly be caused by reduced gene flow. This in turn could be due to the earlier appearance of sexuals of the non host-alternating form in the season (Hille Ris Lambers 1953). Statistically, this idea is substantiated, although the size of the April-June samples from *R. rubrum* makes this conclusion less definitive. An alternative explanation would be to relate the genetic variation to differences in selection related to season (Tomiuk & Wöhrmann 1981; Rhomberg et al. 1985) or simply to host plant.

The mobility of the unique PGI allozyme D of *C. galeopsidis*(Lg) differs only slightly from that of allozyme E of the other *C. galeopsidis* forms. These two allozymes could be distinguished only when tested on the same gel. Therefore, if *C. galeopsidis*-(Lg) has occurred in the samples from the shared host *R. rubrum*, it could be included erroneously in the results.

It is not likely that this may have occurred frequently. Firstly, none of eleven clones from *R. rubrum*, tested for host-alternation in the laboratory, belonged to *C. galeopsidis*(Lg). Secondly, most of the samples from *R. rubrum* (77%) were collected in

July-September, after the migration to *Lamium galeobdolon* (Guldemond, unpublished results).

A few individuals of *C. galeopsidis*(Lg) in the samples from *R. rubrum*, would not seriously influence the difference in allele frequencies observed between the different forms of *C. galeopsidis*. Moreover, correcting for contamination of the samples with *C. galeopsidis*(Lg) would only increase the difference (Table 1.3).

Another pitfall in measuring allele frequencies in aphids is their cyclical parthenogenetic nature. As a consequence they have a clonal population structure which could lead to an underestimation of genetic variation. This might bring into question the significance of the results on PGI polymorphism in the *C. galeopsidis* complex.

There is evidence, however, that this clonal effect on the results, if any, is negligible. Samples of *C. galeopsidis* from one locality usually comprised of individuals from more than one clone. In one sample, even the maximum number of six PGI alleles was found. Further, the data are based on a moderate number (<25) of individuals per sample from a large number of localities and therefore constitute strong evidence for PGI polymorphism.

Consequently, the absence of variation at most of the other enzyme loci cannot be attributed to biased sampling. Because of the low degree of polymorphism generally found in aphids, (e.g. Rhomberg et al. 1985), an electrophoretic study, using few individuals per species, can give a reliable picture of the phylogenetic relationships (Nei 1978).

Phylogenetic trees

The construction of phylogenetic trees on the basis of electrophoretic data is hampered by the lack of agreement on the choice of the best method (Berlocher 1984). Therefore, three different methods were used, each based on different assumptions. Slightly different results were obtained.

The consensus tree, produced with the independent allele model (Figure 1.1C) is, in contrast to the other trees (Figures 1.1A & B), in close agreement with earlier taxonomic conclusions: on morphological grounds the *galeopsidis-alboapicalis* group was placed in a separate genus, *Myzella*, by Börner (1930) and *stachydis*, the most aberrant species in the subgenus *Ampullosiphon* (Eastop & Hille Ris Lambers 1976). This justi-

fies the idea that *C. stachydis* should be considered to be the closest relative (sister group) of all the other *Cryptomyzus* taxa. Nevertheless, the same electrophoretic data also concur with other phylogenetic trees. The use of three outgroup species in this study gives more reliable information on the pleisiomorphic character states.

The use and strength of methods based on distance measures has been discussed by Farris (1981), Nei et al. (1983), Berlocher (1984), Felsenstein (1984) and Hillis (1984). The independent allele model is critisized by Swofford & Berlocher (1987). A disadvantage is its sensitivity to sample size: an allele occurring at a frequency of 0.05 will be missed in about 13% of 20-individual samples and in less then 1% of 50-individual samples. Obviously, too few samples of *C. heinzei*, *C. stachydis* and the *Nasonovia* species were collected to avoid missing rare alleles. With respect to the other taxa, sufficient individuals were sampled in most cases (Table 1.2). Moreover, most enzyme loci in *Cryptomyzus* appear to be monomorphic (Table 1.2) and allele frequencies appeared to be unimportant in differentiating between the taxa at the species level.

A serious theoretical objection to the independent allele model is that it assumes the allele (electromorph) to have a two state character: present or absent. This implies that the lack of an allele can define clades ("absence character" see Figure 1.1C), although actually the allele is present, but only in another condition. This is biologically and phylogenetically illogical.

Consequently, the evidence for the relationship of *C. stachydis* to the other taxa is not strong because it is only based on two "absence characters" (-18, -25, Figure 1.1C). More correctly the locus should be considered to have a multistate character as in the Transformation Series Analysis (Mickevich 1982) or the MANAD method of Swofford & Berlocher (1987). (Both computer programs are not currently available to us).

An advantage of the independent allele model is that the number and distribution of homoplaseous characters and of the possibly less informative "absence characters" can easily be found. Further, the number of shortest, equally probable trees produced by the Wagner parsimony method gives an indication of the reliability of the phylogenetic relationships arrived at. When, on the other hand, genetic distance measures are applied, only one single tree is calculated, whereas no information is presented on almost equally probable trees. Therefore, the position of *stachydis*, *ribis-korschelti* and *heinzei-ballotae* in Figures 1.1A and 1.1B should not be considered too rigidly.

The phylogenetic analysis showed that several taxa are consistently combined, but that no firm conclusions can be drawn about the lower branches of the trees.

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REFERENCES

Avise, J.C. (1983) Protein variation and phylogenetic reconstruction. In: Protein polymorphism: adaptive and taxonomic significance. (ed. by G.S. Oxford and D. Rollinson) pp. 103-130. Academic Press, London and New York.

Berlocher, S.H. (1984) Insect molecular systematics. Ann. Rev. Entomol. **29**, 403-433.

Börner, C. (1930) Beiträge zu einem neuen System der Blattläusen. Arch. Klassif. phylogen. Ent. **I**, 115-194.

Brookes, C.P. & Loxdale, H.D. (1987) Survey of enzyme variation in British populations of *Myzus persicae* (Sultzer) (Hemiptera: Aphididae) on crops and weed hosts. Bull. entomol. Res. **77**, 83-89.

Eastop, V.F. & Hille Ris Lambers, D. (1976) Survey of the world's aphids. Junk Publishers, The Hague.

Eggers-Schumacher, H.A. (1987) Enzyme electrophoresis in biosystematics and taxonomy of aphids. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon & L. Weismann) pp. 63-70. SPB Academic Publishing, The Hague.

Farris, J.S. (1972) Estimating phylogenetic trees from distance matrices. Am. Nat. **106**, 645-668.

Farris, J.S. (1981) Distance data in phylogenetic analysis. In: Advances in cladistics. (ed. by V.A. Funk & D.R. Brooks) pp. 3-23. NY Botanical Garden, New York.

Felsenstein, J. (1984) Distance methods for inferring phylogenies: a justification. Evolution **38**,

16-24.

Ferguson, A. (1980) Biochemical systematics and evolution. Blackie, Glasgow and London.

Furk, C. (1979) Field collections of *Aphis fabae* Scopoli s. lat. (Homoptera: Aphididae) studied by starch gel electrophoresis and isoelectric focussing. Comp. Biochem. Physiol. **62** B, 225-230.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon & L. Weismann) pp. 96-99. SPB Academic Publishing, The Hague.

Heie, O.E. (1979) Revision of the aphid genus *Nasonovia* Mordvilko, including *Kakima* Hottes and Frison, with keys and descriptions of the species of the world (Homoptera: Aphididae). Ent. scand. Suppl. **9**, 1-105.

Hille Ris Lambers, D. (1953) Contribution to a monograph of the Aphididae of Europe V. Temminckia **9**, 1-176.

Hillis, D.M. (1984) Misuse and modification of Nei's genetic distance. Syst. Zool. **33**, 238-240.

Loxdale, H.D., Castañera, P., & Brookes, C.P. (1983) Electrophoretic study of enzymes from cereal aphid populations. I. Electrophoretic techniques and staining systems for characterising isozymes from six species of cereal aphids (Hemiptera: Aphididae). Bull. ent. Res. **73**, 645-658.

Loxdale, H.D., Tarr, I.J., Weber, C.P., Brookes, C.P., Digby, P.G.N. & Castañera, P. (1985) Electrophoretic study of enzymes from cereal aphid populations. III. Spatial and temporal genetic variation of populations of *Sitobion avenae* (F.) (Hemiptera: Aphididae). Bull. ent. Res. **75**, 121-141.

May, B & Holbrook, F.R. (1978) Absence of genetic variability in the green peach aphid, *Myzus persicae* (Hemiptera: Aphididae). Ann. Ent. Soc. Am. **71**, 809-812.

Mickevich, M.F. (1982) Transformation Series Analysis. Syst. Zool. **31**, 461-478.

Mickevich, M.F. & Mitter, C. (1981) Treating polymorphic characters in systematics: a phylogenetic treatment of electrophoretic data. In: Advances in cladistics. (ed. by V.A. Funk & D.R. Brooks). pp. 45-58. NY Botanical Garden, New York.

Narzikulov, M.H. & Daniarova, M.M. (1979) [A new species of aphididae of the genus *Cryptomyzus* Oestl., 1922 (Homoptera: Aphididae) from Tadzhikistan] in russian. Trudy Vses. ent. Obshch. **61**, 42-44.

Nei, M. (1972) Genetic distance between populations. Am. Nat. **106**, 283-292.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**, 583-590.

Nei, M., Tajima, F. & Tateno, Y. (1983) Accuracy of estimated phylogenetic trees from molecular data. II. gene frequency data. J. Mol. Evol. **19**, 153-170.

Nevo, E. (1978) Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. **13**, 121-177.

Odermatt, F. (1981) Die Stärke-Gel-Elektrophorese als Methode zur Artdifferenzierung der "Schwartzen Blattläusen": *Aphis fabae* Scop. und verwandte Arten (Homoptera: Aphididae). J. appl. Ent. **92**, 398-408.

Prager, E.M. & Wilson, A.C. (1976) Congruence of phylogenies derived from different proteins. J. Mol. Evol. **9**, 45-57.

Rhomberg, L.R., Joseph, S. & Singh, R.S. (1985) Seasonal variation and clonal selection in cyclically parthenogenetic rose aphids (*Macrosiphum rosae*). Can. J. Genet. Cytol. **27**, 224-232.

Rogers, J.S. (1972) Measures of genetic similarity and genetic distance. Studies in genetics VII, Univ. Texas Publ. 7213, 145-153.

Shaw, C.R. & Prasad, R. (1970) Starch gel electrophoresis of enzymes. A compilation of recipes. Biochem. Genetics **4**, 297-320.

Simon, J.P., Parent, M.A. & Auclair, J.L. (1982) Isozyme analysis of biotypes and field populations of the pea aphid *Acyrthosiphon pisum*. Entomol. exp. appl. **32**, 186-192.

Singh, R.S. & Rhomberg, L.R. (1984) Allozyme variation, population structure, and sibling species in *Aphis pomi*. Can. J. Genet. Cytol. **26**, 364-373.

Sites, J.W., Bickham, J.W., Pytel, B.A., Greenbaum, I.F. & Bates, B.A. (1984) Biochemical characters and the reconstruction of turtle phylogenies: relationships among batagurine genera. Syst. Zool. **33**, 137-158.

Sneath, P.H.A. & Sokal, R.R. (1973) Numerical taxonomy. Freeman, San Fransisco.

Steiner, W.W.M., Voegtlin, D.J. & Irwin, M.E. (1985) Genetic differentiation and its bearing on migration in North American populations of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae). Ann. Ent. Soc. Amer. **78**, 518-525.

Swofford, D.L. & Berlocher, S.H. (1987) Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. **36**, 293-325.

Takada, H. (1986) Genotypic composition and insecticide resistance of Japanese populations of *Myzus persicae* (Sultzer) (Homoptera: Aphididae). J. appl. Ent. **102**, 19-38.

Tomiuk, J. & Wöhrmann, K. (1981) Changes of genotypic frequencies at the MDH-locus in populations of *Macrosiphum rosae* (L.) (Hemiptera: Aphididae). Biol. Zbl. **100**, 631-640.

Tomiuk, J. & Wöhrmann, K. (1983) Enzyme polymorphism and taxonomy of aphid species. Z. zool. Syst. Evolut.-forsch. **21**, 266-274.

Tomiuk, J. & Wöhrmann, K. (1984) Genotypic variability in natural populations of *Macrosiphum rosae* (L.) in Europe. Biol. Zbl. **103**, 113-122.

Tomiuk, J., Wöhrmann, K. & Eggers-Schumacher, H.A. (1979) Enzyme patterns as a characteristic for the identification of aphids. J. appl. Ent. **88**, 440-446.

Weber, G. (1985) Genetic variability in host plant adaptations of the green peach aphid, *Myzus persicae*. Entomol. exp. appl. **38**, 49-56.

Wool, D., Bunting, S. & van Emden, H.F. (1978) Electrophoretic study of genetic variation in British *Myzus persicae* (Sulz.) (Hemiptera, Aphididae). Biochem. Genet. **16**, 987-1006.

2 Host plant relationships of the aphid genus *Cryptomyzus*

ABSTRACT

Host plant relationships in the aphid genus *Cryptomyzus* were studied by field sampling and laboratory experiments. Host plant suitability and host plant preference were assessed using exules, females of the parthenogenetic summer generations, of closely related taxa. The differences found in host plant suitability and host plant preference between several closely related taxa justify their statuses as separate species. Previously unknown life cycles and host plant relationships were found. Selective host plant preference was found to favour the colonization of suitable host plants and to be correlated with reproductive performance on that host plant. The involvement of induction of performance in *C. galeopsidis* could not be demonstrated experimentally.

key words: *Cryptomyzus*, aphid, host plant preference, host plant suitability, reproductive performance, induction, biosystematics.

INTRODUCTION

In the overwhelming diversity of relationships between herbivorous insects and their host plants, the number of suitable host plants for different species appears to vary greatly. Aphids have developed close associations with their host plants. Differences in host plant relations and life cycle can therefore be useful characteristics for determining the taxonomic status and evolutionary relationships of aphid species.

Almost all aphid species (99%) feed and reproduce on only a limited number of taxonomically related host plants (Eastop 1973). Closely related aphid taxa, which often cannot be distinguished morphologically, may have different host plant relationships. Several so-called subspecies and biotypes of *Acyrthosiphon pisum* (Harris) are characterized mainly by their specific host plants (Müller 1971, 1980; Müller & Steiner 1985). In the *Aphis fabae* Scopoli species group (Iglisch 1968) a host plant test is the only way to discriminate the different taxa.

Life cycles may also differ between closely related aphid species. Some are host-alternating (dioecious) species which migrate from the primary to the secondary host plant in spring and return in the autumn. Others are non host-alternating (monoecious) species and remain on the same host plant species during their whole life cycle. This implies that most aphid species occupy a specific niche, which is determined by its host plants and type of life cycle.

The evolutionary relationships between these features were studied using the genus *Cryptomyzus*. This genus is composed of a group of closely related species, some of uncertain taxonomic status, which shows the whole array of different combinations of host plant relations and life cycles. The biology of *Cryptomyzus* was described by Hille Ris Lambers (1953) and the nomenclature follows Eastop & Hille Ris Lambers (1976). The primary host plants are species of *Ribes*, e.g. black and red currant and the secondary host plants all belong to the Labiatae.

This study requires the introduction of some specific forms. Of *C. alboapicalis* (Theobald) two forms were found, which occur monoeciously on *Lamium album* or on *L. maculatum*. These two forms are referred to as *C. alboapicalis*(L. album) and *C. alboapicalis*(L. maculatum). The host-alternating *C. galeopsidis* (Kaltenbach) is found on two different secondary host plants (Guldemond 1987). These forms are referred to as *C. ga-*

leopsidis(G. tetrahit), migrating to *Galeopsis tetrahit* and *C. galeopsis*(L. galeobdolon), migrating to *L. galeobdolon* (see fold-out page). The primary hosts, if any, of *C. heinzei* Hille Ris Lambers, *C. ballotae* Hille Ris Lambers and *C. leonuri* Bohzko are unknown.

The host plant relationships were assessed both on the basis of field collections, and on host plant preference and host plant suitability tested in the laboratory. The latter parameter is commonly measured by the reproductive capacity over a certain period of time and is also referred to as reproductive performance (see Thompson 1988).

In the present study the following questions are addressed. What are the life cycles and the host plant relationships of the various *Cryptomyzus* taxa and can host range be used to discriminate between the taxa? Is host plant preference correlated with performance? This paper describes the experimental findings on host plant suitability and preference, mainly of exules, which are females of the parthenogenetic summer generations living on secondary host plants. Host plant relationships of the sexual morphs and their implications for reproductive isolation between closely related taxa will be discussed in a separate paper (Chapter 3).

MATERIAL AND METHODS

During 1983-1988 over 200 samples of *Cryptomyzus* were collected in the Netherlands and other European countries. A list of localities and host plants is available on request (Appendix). The clones used in the laboratory experiments all originated in the Netherlands, except for one clone of *C. korschelti* Börner (France), *C. ballotae* (Great Britain), *C. heinzei* (West Germany), *C. stachydis* (Heikinheimo) (Finland) and two clones of *C. alboapicalis*(L. album) (France and Great Britain). All clones were reared on field collected plants. Perennial, herbaceous plants were collected once and propagated by means of shoot cuttings, whereas annuals were grown from field collected seeds. *Ribes* species were obtained from commercial plant growers. Host plant species, presumed to be characteristic for the various taxa, were selected on the basis of records in the literature (Hille Ris Lambers 1953, Shaposhnikov 1967).

Stock cultures were kept in a glasshouse at 20 ± 2°C, 18 hours light, relative humidity 70-80%. Experiments were carried out under similar conditions. Both experiments

on sexuals and the breeding of them were carried out under short day conditions (10 hours light) in a growth chamber.

In all experiments, only young and vigorous individuals were used. In general, experiments with exules were conducted with the winged morph, as this is the dispersing morph which selects a new host plant under natural conditions. Some clones did not produce sufficient winged females and in these cases wingless were used for the experiments.

Host plant suitability was measured in terms of survival and production of adult aphids on a test host plant over a period of seven days (P7). The reproduction on the test plant was compared with that on the culture host plant. Survival from birth to the start of reproduction was also measured. A host plant is defined as suitable when it sustains growth and reproduction. The plants used in the experiments were in those early stages of development up to the appearance of flower buds.

Host plant preference experiments were carried out in a cage, 31 x 22 x 40 cm., with dark walls and a fine gauze cover illuminated centrally from above. In each trial two potted plants of different species were surrounded by moist sand to the level of their top edges. Only young, growing plants were used, cut to equal size when necessary (see Åhman et al. 1985). A choice experiment was started by releasing up to 120 aphids from a glass tube placed between the two plants. After 24 hours the distribution of individuals and their offspring on each plant was recorded (Rautapää 1970; Leather & Dixon 1982). Individuals found on the cage and the bottom were scored "no choice". Different morphs were tested separately.

RESULTS

Host plant relationships

Both field collections and the results of host plant suitability experiments have provided new information on host plant relationships for several *Cryptomyzus* species. These results are summarized in Table 2.1 and Figure 2.1. The heteroecious life cycle of *C. heinzei* was confirmed by its discovery on *Ribes alpinum* in spring (pers. comm. H.A. Eggers-Schumacher). Transfer experiments show that its migrants thrive on *Stachys*

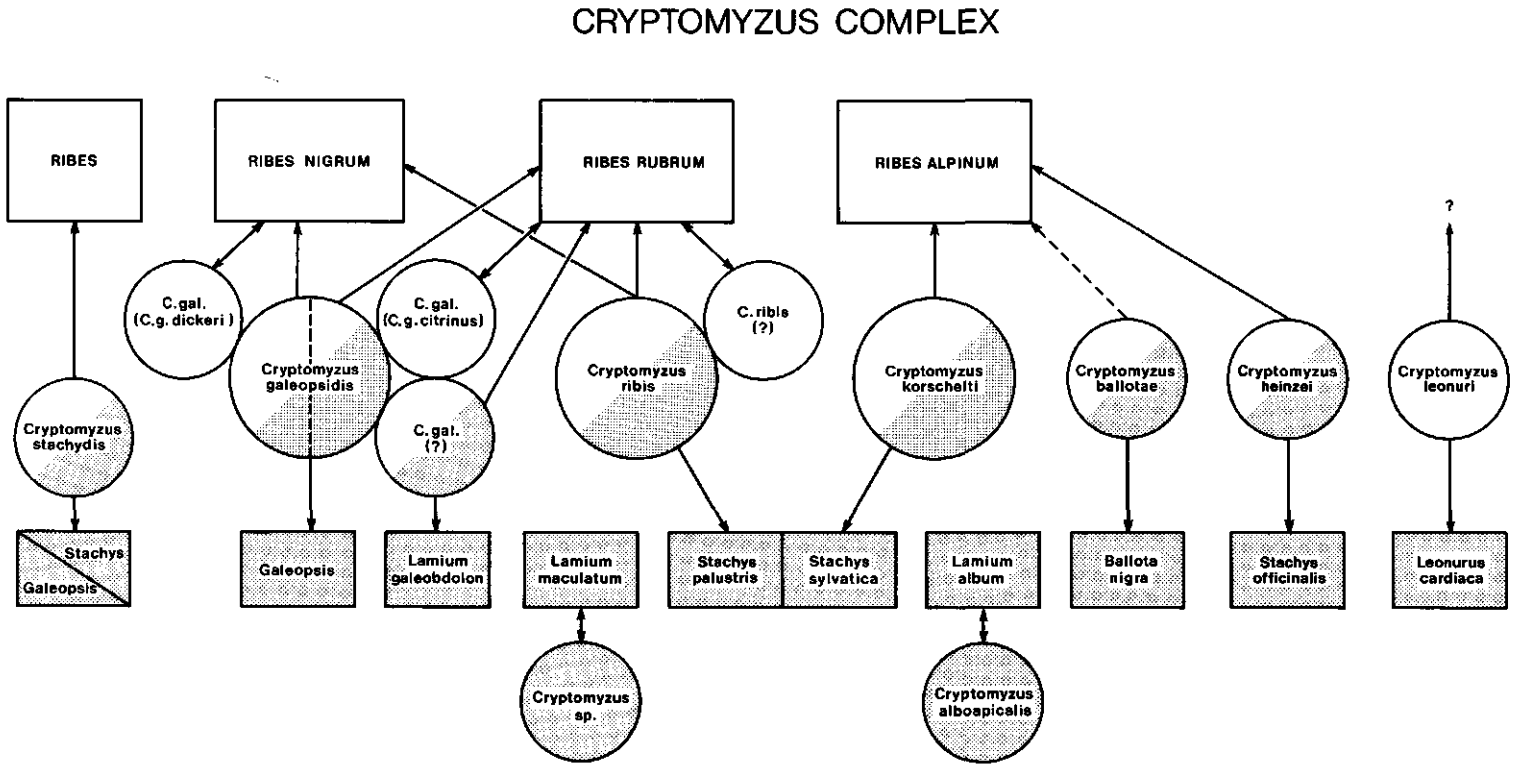

Figure 2.1 Host plant relationships of *Cryptomyzus* taxa (circles). In boxes on the top row the primary host plants and in the bottom row the secondary host plants.

officinalis and in laboratory tests oviparae only develop on *R. alpinum* (Table 2.1). The primary host plant of *C. ballotae* is unknown, but tests indicate that its oviparae only develop on *R. alpinum* (Table 2.1). However, the species has never been found on *R. alpinum* in the field.

The data on host plant suitability (Table 2.1) show that each *Cryptomyzus* taxon is characterized by a specific combination of secondary host plants. On the other hand, most of these plants are suitable for only one specific aphid species. Exceptions to this rule are two annual *Lamium* species, *L. amplexicaule* and *L. purpureum*, which are suitable for all tested *Cryptomyzus* taxa, and *Galeopsis tetrahit* which is suitable for all except the two forms of *C. alboapicalis*. Unlike earlier reports (Hille Ris Lambers 1953) *C. ribis* (L.) not only performs better on *Stachys palustris* compared with *S. sylvatica* (Table 2.1), but is also found more frequently on this plant ($X^2_{(1)}$=5.40, n=15, P<0.05). *S. sylvatica* is the main host plant of *C. korschelti*.

C. ribis and *C. ballotae* show a broad range of suitable host plants with 9 plant species out of 13. The incomplete data on *C. stachydis* indicate that it is an exceptional species, which may live on even more hosts than the plant species studied. *C. alboapicalis*(L. album), on the other hand, has a restricted host range and is able to live on only 3 of the host plant species, whereas *C. alboapicalis*(L. maculatum) and *C. galeopsidis*(G. tetrahit) may live on only 4 plant species. A diagram of all characteristic host plant relationships known at present is presented in Figure 2.1.

Table 2.1 Host plant suitability for taxa of *Cryptomyzus*. Suitability (S) is indicated as:
+ = mean reproduction ≥50% of reproduction on the culture host plant (*) and offspring reach adult stage and reproduce in all tests.
± = mean reproduction <50% and occasionally reproducing offspring.
- = mean reproduction <50% and no reproducing offspring.
. = no measurements.
° = data are based on measurements with a reduced number of clones.
Field samples/ literature records (F/L):
+ = field collections in the period 1983-1988 and/or recorded in literature or in the collection of the British Museum (Natural History).
- = no such data found.
In case of no reproduction at least 50 individuals per clone were tested; in case of reproduction at least two experiments with 10 females each were performed; signs in brackets indicate that fewer numbers were tested. Winged females were used, except in *C. stachydis* where only wingless females were available.

		korschelti	*ribis*	*heinzei*	*ballotae*	*alboapicalis* (La)	*alboapicalis* (Lm)	*galeopsidis* (Lg)	*galeopsidis* (Gt)	*stachydis*
number of clones		2	2	1	3	3	2	2	2	1
secondary host plants										
Lamium	S	+±	+	.	+	+	+°	+	+	(+)
amplexicaule L.	F/L	+	+	-	+	+	-	-	+	-
Lamium	S	+±	+	.	+	+	+	+	+	.
purpureum L.	F/L	+	+	-	-	+	-	-	+	-
Lamium galeob-	S	-	-	-	-	-	-	*	-	-
dolon (L.) L.	F/L	-	-	-	-	-	-	+	-	-
Lamium	S	-	-	-	+	*	±-	±-	-	(+)
album L.	F/L	+	-	-	+	+	-	-	+	+
Lamium	S	-	±	-	±	-	*	+±	-	-
maculatum L.	F/L	-	+	-	-	+	+	-	+	-
Galeopsis	S	+	+	±	+±(-)	-	-	±	*	+
tetrahit L.	F/L	+	+	-	-	-	-	-	+	+
Stachys	S	*	±	-	-	-	-	-	-	*
sylvatica L.	F/L	+	+	-	-	-	-	-	-	+
Stachys	S	±-	*	-	-	-	-	-	-	(+)
palustris L.	F/L	-	+	-	-	-	-	-	-	-
Stachys	S	+	+	-	±-	-°	-	-	-	.
annua (L.) L.	F/L	-	+	-	-	-	-	-	-	-
Stachys offici-	S	-	±	*	±°	-°	-	-	-	.
nalis (L.) Trev.	F/L	-	+	+	-	-	-	-	-	-
Ballota	S	-	-	-	*	-	-	-	-	(-)
nigra L.	F/L	+	-	-	+	+	-	-	+	-
Leonurus	S	-	+±	.	(+)°	-°	.	.	-°	(-)
cardiaca L.	F/L	-	+	-	+	-	-	-	-	-
Satureja vulga-	S	-	-	-	-	-	-	-	-	.
ris (L.) Fritsch	F/L	-	-	+	-	-	-	-	-	-
Veronica	S	.	.	.	.	-°	-	-	±+	.
agrestis L.	F/L	-	-	-	-	-	-	-	+	-
primary host plants										
Ribes	S	-	+	-	-°	.	.	+	+-	.
rubrum L.	F/L	+	+	-	-	-	-	-	+	+
Ribes	S	-	+	-	-	.	.	-	+-	.
nigrum L.	F/L	+	+	-	-	-	-	-	+	-
Ribes	S	+	-	+	+	.	.	-	-	.
alpinum L.	F/L	+	+	+	-	-	-	-	+	-

Host plant suitability for exules

The two forms of *C. alboapicalis* show differences in their performance: *C. alboapicalis*-(L. album) does not reproduce on *L. maculatum* and *C. alboapicalis*(L. maculatum) reproduces only occasionally on *L. album* (Table 2.2). Of the two forms of *C. galeopsidis*, *C. galeopsidis*(G. tetrahit) reproduces on *Galeopsis tetrahit* and *Veronica agrestis*. In contrast, *V. persica* Poir. is not accepted by wingless females of two clones. *C. galeopsidis*(L. galeobdolon) accepts *Lamium galeobdolon*, *L. maculatum* and sometimes *L. album*

Table 2.2 Host plant suitability for winged and wingless females (exules) of *C. galeopsidis*(L. galeobdolon) and *C. galeopsidis*(G. tetrahit), and *C. alboapicalis*(L. album) and *C. alboapicalis*(L. maculatum). Number of experiments (exp), number of tested individuals (N), their survival after 7 days (S) and mean number of larvae produced per female in 7 days (P7/♀).

taxon	morph	nr clones	exp	N	S	P7/♀ ± sd
C. alboapicalis		on *Lamium maculatum*				
(La)	winged	8	21	554	0	0
(Lm)	winged	2	7	69	0.54	5.8 ± 1.3
		on *Lamium album*				
(La)	winged	4	12	122	0.58	9.5 ± 2.5
(Lm)	winged	2	6	124	0.05	0.0 ± 0.0
	wingless	3	6	223	0.02	0.2 ± 0.4
C. galeopsidis		on *Galeopsis tetrahit*				
(Lg)	winged	4	17	214	0.06	0.4 ± 0.9
	wingless	6	19	337	0.14	2.2 ± 3.8
(Gt)	winged	2	9	91	0.33	12.4 ± 3.3
		on *Lamium galeobdolon*				
(Lg)	winged	2	9	77	0.61	10.0 ± 3.0
	wingless	2	8	40	0.78	32.2 ± 4.4
(Gt)	winged	7	15	536	0	0
		on *Veronica agrestis*				
(Lg)	winged	2	2	20	0	0
	wingless	3	6	64	0	0
(Gt)	winged	2	4	45	0.18	6.7 ± 2.2
		on *Lamium maculatum*				
(Lg)	winged	2	7	66	0.16	4.8 ± 3.9
	wingless	3	9	135	0.20	6.7 ± 4.3
(Gt)	winged	4	8	230	0	0
		on *Lamium album*				
(Lg)	winged	2	13	146	0.04	0.8 ± 1.5
	wingless	3	8	134	0	0
(Gt)	winged	4	5	154	0	0

Table 2.3 Host plant preference of females (exules) of *C. galeopsidis*(G. tetrahit) and *C. galeopsidis*(L. galeobdolon) for *Galeopsis tetrahit* and *Lamium galeobdolon*. Significant levels using a X^2-test, 1 degree of freedom, are: **P*<0.05, ***P*<0.01, ****P*<0.001.

clone	morph	nr exp	*G. tetrahit*	*L. galeobdolon*	no choice	$X^2_{(1)}$
C.galeopsidis(Gt)						
G62	winged	3	107	12	10	75.84***
G61	winged	3	138	2	0	132.11***
315	winged	2	108	3	2	99.32***
303	winged	2	148	2	7	142.11***
342	winged	3	147	2	11	141.11***
C. galeopsidis(Lg)						
237	winged	3	4	57	0	46.05***
	wingless	3	20	137	5	87.19***
227	wingless	3	16	105	1	65.46***
205	wingless	2	1	92	1	89.04***

and *G. tetrahit*, but on the latter two plants its production of offspring was much lower than on the field host plant *L. galeobdolon* (Table 2.2, for winged and wingless females Mann-Whitney U=0, P<0.001, two tailed). In addition, these cultures were unable to maintain themselves and eventually failed. Furthermore, in host plant preference experiments the forms of *C. galeopsidis* reveal a significant preference for their natural host plant (Table 2.3). Thus, selective host plant preference favours the colonization of suitable host plants.

Aphids were reared continuously on the same host plant species. In order to determine if this had caused induction of host plant preference (Dethier 1982), two clones of *C. galeopsidis*(L. galeobdolon) were forced to live on the occasionally suitable host *G. tetrahit*. In two cases out of four the aphids failed to establish themselves and aphids in the other two cases were initially successful, but subsequently died out. The first generation females which matured on *G. tetrahit* did not yield a single larva (Table 2.4). As it proved impossible to rear *C. galeopsidis*(L. galeobdolon) continuously on *G. tetrahit*, it is unlikely that induction is involved in host plant preference shown by the two forms of *C. galeopsidis*.

Table 2.4 Survived individuals (S) and fecundity over 7 days (P7) of the wingless females of two *C. galeopsidis*(L. galeobdolon) clones on *L. galeobdolon* (culture host) and *G. tetrahit*. Cultures marked with an asterix * provided females for the second generation on *G. tetrahit*.

clone				1st generation			2nd generation		
	N	S	P7	N	S	P7	N	S	P7
	Lamium galeobdolon			*Galeopsis tetrahit*					
237	5	5	153	10	0	2	5	0	0
	5	5	163	10	0	0	5	0	0
	5	4	166	10	0	0	10	0	0
	5	3	199	10	2	50*			
205	5	3	119	10	0	0	10	0	0
	5	4	160	10	0	0	10	0	0
	5	4	169	10	3	72*	10	0	0
	5	3	159	10	0	12	10	0	0

DISCUSSION

Host plant suitability and host plant preference

The data on host plant suitability were obtained from experiments using only a few clones per taxon. One might, therefore, question whether the results are representative. Indeed, small differences in host plant suitability have been reported for different clones of polyphagous species like *Myzus persicae* Sulzer (Weber 1985a & 1986), *Metapolophium dirhodium* (Walker) (Weber 1985b) and *Sitobion avenae* (F.) (Weber 1985c). Even large differences in performance within a species were found (e.g. Müller 1983; Müller & Steiner 1985), and the authors sometimes decide to consider these forms as "subspecies" (e.g. Müller 1986). Because the secondary host plant preferences of *Cryptomyzus* species, as determined in this study, cover those known from the literature (Table 2.1) there is no evidence for biased results.

In the study of life cycles of *Cryptomyzus* species, the primary host range was also assessed. A wider range is reported in the literature than was found in this study. Because it is not always indicated which morphs were found, several data may refer only to autumn migrants (gynoparae) or males. This does not unequivocally prove that these host plants are suitable hosts. Restricting the records to plants on which oviparae or

fundatrices are found might rather give a more limited host range than our results do. The range of suitable host plants found in this study is thus reliable for the characterization of the different taxa.

Host plant preference and suitability could have been influenced by previous induction (Dethier 1982; Jermy 1987). This phenomenon has been demonstrated to occur in several aphid species (Lowe 1973; Hubert-Dahl 1975; Schweissig & Wilde 1979). It is only likely to manifest itself when a population can live relatively well on at least two host plants. The closely related taxa *C. galeopsidis*(L. galeobdolon) and *C. galeopsidis*(G. tetrahit) exhibit a striking difference in host plant suitability: in a number of cases *C. galeopsidis*(L. galeobdolon) could live on the host plant of *C. galeopsidis*(G. tetrahit), but the reverse is impossible. An attempt to induce *C. galeopsidis*(L. galeobdolon) to reproduce on *Galeopsis tetrahit* failed because its survival on this plant was too low (Table 2.4). The observed asymmetry of host plant suitability between the two forms of *C. galeopsidis* is possibly due to a phylogenetic constraint (Futuyma 1983), indicating that *C. galeopsidis*(L. galeobdolon) evolved from an ancestor which lived on *Galeopsis* and has retained the capacity to live on the "ancestral" host plant to a certain extent. The same reasoning may be applicable to the two forms of *C. alboapicalis.*

Host plant specifity

Just as there are polyphagous aphid species which may live on many, taxonomically unrelated plants, there are also "poly-suitable" plants, like the annual *Capsella bursa-pastoris* L., which are suitable for many, unrelated aphid species (Eastop 1973). Similarly, it appears that the annuals *Lamium purpureum* and *L. amplexicaule* are suitable for all *Cryptomyzus* taxa (Table 2.1), whereas only one or a few *Cryptomyzus* taxa can survive on the perennials *Lamium galeobdolon* and *L. album.* However, not all annuals are suitable, like *Stachys annua* which is only suitable for two species of *Cryptomyzus.* The fact that *L. purpureum* and *L. amplexicaule* are suitable for all *Cryptomyzus* species is possibly due to the fact that these plants complete their life cycle early in spring, before aphids migrate from their primary host plant (Berryman 1988). Under natural conditions the aphids may arrive only after the plant has completed its life cycle, and therefore has probably developed only a weak defence system. Because it has been demonstrated that aphid infestation can reduce the survival of seedlings and young plants

of annuals (Ernst 1987), it is worth pointing out that, in contrast, *S. annua* develops in full summer and is therefore probably better protected against aphid attacks, among other things.

In his broad survey on host plant relationships of all aphid families, Eastop (1973) showed that in general host-alternating species accept more secondary host plants than monoecious species. From the present study this also appears to hold for species within one genus. For the host-alternating taxa of *Cryptomyzus* 6 of the tested plant species were suitable on average, whereas only 3 and 4 were suitable for the two monoecious taxa. This phenomenon might be caused by the necessity of finding a mating partner, the chance of which is increased when only a limited number of hosts is used (Ward 1987). Host-alternating species with many secondary host plants also have a limited number of primary hosts, where the sexes meet.

Taxonomic implications of host plant relationships

The genetic basis of reproductive performance has been demonstrated for several insect groups (Futuyma & Peterson 1985; Müller 1985) and this behaviour can therefore be used as an ecological character to discriminate between closely related taxa. Exules of *C. galeopsidis*(L. galeobdolon) and *C. galeopsidis*(G. tetrahit) show differences in performance on host plants and the same applies to the two forms of *C. alboapicalis*. This indicates the presence of disjunct gene pools and suggests the existence of separate species, which is corroborated by electrophoretic data (Guldemond & Eggers-Schumacher 1989).

Further, *C. alboapicalis* has also been reported from *Ballota nigra* (Hille Ris Lambers 1953 and collection of the British Museum). Attempts to transfer this aphid to *B. nigra* using five clones of *C. alboapicalis*(L. album) and three clones of *C. alboapicalis*-(L. maculatum) were all unsuccessfull. Another genotype may exist which can survive on *B. nigra*. If there is a form that survives exclusively on *B. nigra*, it might be that another species is involved, because all examined clones of *C. alboapicalis* are strictly monoecious.

This study reveals that the primary host plant of *C. heinzei* is *Ribes alpinum*. The data is conflicting about the secondary host. *C. heinzei* was collected only once on *Satureja vulgaris*, in Germany (Hille Ris Lambers 1953). However, it has been collected

from *Stachys officinalis* in Spain, West-Germany and Hungary (Appendix) and in Czechoslovakia (collection of the British Museum). My laboratory experiments show that *C. heinzei* (also collected in Germany) can not survive on *Satureja*, but can successfully be cultured on *S. officinalis*. It remains possible that *Satureja* is occasionally used as a host plant or that a misidentification is involved. Within the Labiatae, *Satureja* is only distantly related to the other secondary host plants of *Cryptomyzus*, which on the other hand form a closely related group and includes *S. officinalis* (El-Gazzar & Watson 1970). This also may indicate that *S. officinalis* is the most probable host plant of *C. heinzei*.

Many authors give a subspecific status to closely related, morphologically indistinguishable aphids, which differ in characteristic host plant (see e.g. Eastop & Hille Ris Lambers 1976; Müller 1986). This use of the well defined term subspecies should be avoided, because subspecies exclude each other geographically (Mayr 1969). Probably, in these cases, different species are involved, as can be shown using an electrophoretic approach (e.g. Eggers-Schumacher 1987) or by hybridization experiments (Müller 1985). From the present study it appears that the host plant range may also show marked differences between closely related aphid taxa and therefore can be used, in combination with other characters, to define species.

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REFERENCES

Åhman, J., Weibull, J. & Petterson, J. (1985) The role of plant size and plant density for host finding in *Rhopalosiphum padi* (L.) (Hem.: Aphididae). Swed. J. Agric. Res. **15**, 19-25.

Berryman, A.A. (1988) Towards a unified theory of plant defence. In: Mechanisms of woody

plant defenses against insects. Search for pattern. (ed. by W.J. Mattson, J. Levieux and C. Bernard-Dagan), pp. 39-56. Springer Verlag, New York.

Dethier, V.G. (1982) Mechanism of host-plant recognition. Entomol. exp. appl. **31**, 49-56.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp. Roy. Ent. Soc. London **6**, London.

Eastop, V.F. & Hille Ris Lambers, D. (1976) Survey of the world's aphids. Junk Publishers, The Hague.

Eggers-Schumacher, H.A. (1987) Enzyme electrophoresis in biosystematics and taxonomy of aphids. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 63-70. SPB Academic Publishing, The Hague.

El-Gazzar, A. & Watson, L. (1970) A taxonomic study of Labiatae and related genera. New Phytol. **69**, 451-486.

Ernst, W.H.O. (1987) Impact of the aphid *Aulacorthum solani* Kltb. on growth and reproduction of winter and summer annual life forms of *Senecio sylvaticus* L.. Acta Oecol. **8**, 537-548.

Futuyma, D.J. (1983) Selective factors in the evolution of host choice by phytophagous insects. In: Herbivorous insects: host seeking behaviour and mechanisms. (ed. by S. Ahmad), pp. 227-244. Academic Press, New York.

Futuyma, D.J. & Peterson, S.C. (1985) Genetic variation in the use of resources by insects. Ann. Rev. Entomol. **30**, 217-238.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 96-99. SPB Academic Publishing, The Hague.

Guldemond, J.A. & Eggers-Schumacher H.A. (1989) Biosystematics of the aphid genus *Cryptomyzus* (Homoptera, Aphididae): an electrophoretic analysis. Z. zool. Syst. Evolut.-forsch. **27**, 14-25.

Hille Ris Lambers, D. (1953) Contribution to a monograph of the Aphididae of Europe V. Temminckia **9**, 1-176.

Hubert-Dahl, M.L. (1975) Änderung des Wirtswahlverhaltens dreier Biotypen von *Acyrthosiphon pisum* Harris nach Anzucht auf verschiedenen Wirtspflanzen. Beitr. Ent. **25**, 77-83.

Iglisch, I. (1968) Über die Entstehung der Rassen der "Schwartzen Blattläuse" (*Aphis fabae* Scop. und verwante Arten), über ihre phytopathologische Bedeutung und über die Aussichten für erfolgversprechende Bekämpfungsmassnamen (Homoptera: Aphididae). Mitt. Biol. Bund-

Anst. Ld- u. Forst., Berlin-Dahlem, **131**, 1-34.

Jermy, T. (1987) The role of experience in the host selection of phytophagous insects. In: Perspectives in chemoreception and behavior. (ed. by R.F. Chapman, E.A. Barnays & J.G. Stoffolano, Jr.), pp.143-157, Springer-Verlag, New York.

Leather, S.R. & Dixon, A.F.G. (1982) Secondary host preference and reproductive activity of the bird cherry-oat aphid *Rhopalosiphum padi*. Ann. appl. Biol. **101**, 219-228.

Lowe, H.J.B. (1973) Variation in *Myzus persicae* (Sulz.) (Hemiptera, Aphididae) reared on different host plants. Bull. ent. Res. **62**, 549-556.

Mayr, E. (1969) Principles of systematic zoology. pp. 41-42. McGraw-Hill, New York.

Müller, F.P. (1971) Isolationmechanismen zwichen sympatrischen bionomischen Rassen am Beispiel der Erbsenblattlaus *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae). Zool. Jb. Abt. Syst. Ökol. Geogr. Tiere, **98**, 131-152.

Müller, F.P. (1980) Wirtspflantzen, Generationsfolge und reproductive Isolation intraspecifiker Formen von *Acyrthosiphon pisum*. Entomol. exp. appl. **28**, 145-157.

Müller, F.P. (1983) Untersuchungen über Blattläuse der Gruppe *Acyrthosiphon pelargonii* im Freiland-Insektarium. Z. angew. Zool. **70**, 351-367.

Müller, F.P. (1985) Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. Biol. Zbl. **104**, 225-237.

Müller, F.P. (1986) The role of subspecies in aphids for applied entomology. J. appl. Ent. **101**, 295-303.

Müller, F.P. & Steiner, H. (1985) Das Problem *Acyrthosiphon pisum* (Homoptera: Aphididae). Z. angew. Zool. **72**, 317-334.

Rautapää, J. (1970) Preference of cereal aphids for various cereal varieties and species of Gramineae, Juncaceae and Cyperaceae. Ann. Agric. Fenn. **9**, 267-277.

Schweissing, F.C. & Wilde, G. (1979) Predisposition and nonpreference of the greenbug for certain host plants. Environ. Entomol. **8**, 1070-1072.

Shaposhnikov, Ch.G. (1967) in: Key to the insects of the european USSR, vol. 1. (ed. by Bei-Bienko), pp. 787-788. Israel Program for Scientific Translation, Jerusalem.

Thompson, J.N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. exp. appl. **47**, 3-14.

Ward, S.A. (1987) Cyclical parthenogenesis and the evolution of host range in aphids. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 39-44. SPB Academic Publishing, The Hague.

Weber, G. (1985a) Genetic variability in host plant addaptation of the green peach aphid, *Myzus persicae*. Entomol. exp. appl. **38**, 49-56.

Weber, G. (1985b) On the ecological genetics of *Metapolophium dirhodium* (Walker) (Hemiptera, Aphididae). J. appl. Ent. **100**, 451-458.

Weber, G. (1985c) On the ecological genetics of *Sitobion avenae* (F) (Hemiptera, Aphididae). J. appl. Ent. **100**, 100-110.

Weber, G. (1986) Ecological genetics of host plant exploitation in the green peach aphid, *Myzus persicae*. Entomol. exp. appl. **40**, 161-168.

3 Choice of host plant as factor in reproductive isolation of the aphid genus *Cryptomyzus* [1]

ABSTRACT

Host plant preference experiments were conducted with closely related taxa of the aphid genus *Cryptomyzus*. Males, and presexual morphs (sexuparae and gynoparae), were used to determine the impact of host plant choice on reproductive isolation. In the case of host-alternating species these morphs are migratory and so will select the host plant. Host plant preference of two closely related taxa of *C. alboapicalis* was found to promote their reproductive isolation. The preference of sexuparae of these monoecious taxa was more pronounced than that of the males. Host plant preference and subsequent production of oviparae showed that *C. galeopsidis* consists of two host races restricted to *Ribes rubrum* and *R. nigrum*, respectively. The existence of clones, intermediate in their preference and reproductive performance on these plants, suggests that hybridization occurs.

key words: *Cryptomyzus*, aphid, preference, reproductive isolation, host race, sibling species, assortative mating.

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[1]Ecological Entomology (1990, in press)

INTRODUCTION

The life cycle of most aphid species is characterized by parthenogenetic propagation in spring and summer, and a sexual phase in autumn. In late summer, host-alternating (dioecious) species produce specialized asexual female morphs on their secondary ("summer") host. These gynoparae migrate to the primary ("winter") host plant and then start to produce oviparae. The oviparae mate with males, which have also arrived from the secondary host. They then lay overwintering diapause eggs. In species that do not alternate between hosts (monoecious), oviparae and males are produced by sexuparae which appear in autumn but do not migrate (Dixon 1985; for definitions of aphid morphs, see Hille Ris Lambers 1966).

Gene flow between various clones of aphids can only occur when they reproduce sexually on the primary host. So differences in host preference shown by the presexual morphs (sexuparae and gynoparae, which produce the sexuals) and males of closely related forms may lead to reproductive isolation between these forms. Several other mechanisms of reproductive isolation have been described for aphids by Müller (1985). Premating mechanisms, such as differences in reproductive performance on various host plants, prevent hybridization between closely related monoecious forms, and post-mating mechanisms, such as hybrid inferiority or sterility, reduce or stop gene flow.

One must distinguish between host plant preference, the choice during the initial phase of settlement on a host, and reproductive performance, which describes survival and reproduction after settlement (e.g. Thompson 1988). Experiments on host plant preference have led to the discovery of sibling species (Müller & Hubert-Dahl 1979), and biotypes or subspecies (Müller 1983). A shift in host preference is considered to be a first step in the colonization of a new, previously unused host plant (Futuyma & Peterson 1985; Jermy 1987) and in the process of speciation of the insect.

The host preferences of aphid presexuals have been determined only rarely (Müller 1958; Dixon 1971). Kennedy et al. (1959a, b) studied their behaviour under field conditions, but to my knowledge, the host choice of males has not been tested in the laboratory.

The genus *Cryptomyzus* Oestlund comprises closely related species with different life cycles and host plants and is therefore suitable for studying reproductive isolation

between closely related and host-specific forms. The biology and taxonomy of this group has been described by Hille Ris Lambers (1953) with additional information by Guldemond (1987).

The present study addresses the question: Do differences in the host plant preference of presexual morphs and males contribute to the reproductive isolation of closely related *Cryptomyzus* taxa? To answer this question the host plant choice of these morphs of several clones was examined. The results revealed considerably more evolutionary differentiation between these forms than was known previously.

MATERIAL AND METHODS

Three species of *Cryptomyzus* were studied and each species comprise two or more host-specific forms. The host plant relationships and life cycle of two species are shown in Figure 3.1 (fold-out page behind). The monoecious *C. alboapicalis* (Theobald) has forms on *Lamium album* L. and *L. maculatum* L., referred to as *C. alboapicalis*(L. album) and *C. alboapicalis*(L. maculatum), respectively. In *C. galeopsidis* (Kaltenbach), one form alternates between the hosts *R. rubrum* and *L. galeobdolon* (L.)L. and is here designated *C. galeopsidis*(L. galeobdolon). Two other forms share the secondary host plant *Galeopsis tetrahit* L., but live on different primary hosts, *Ribes rubrum* L. and *R. nigrum* L.. Both forms are designated *C. galeopsidis*(G. tetrahit) with an indication of their primary host. Finally, the species *C. ribis* (L.) uses as primary hosts both *R. rubrum* and *R. nigrum.*

Clones of these taxa were collected in the Netherlands and were reared on plants collected in the field. Perennial herbaceous plants were collected once and propagated by means of shoot cuttings in the greenhouse, whereas annuals were grown from seeds collected in the field. The *R. rubrum* cultivars 'Rovada' and 'Fay Prolific' and the *R. nigrum* cultivar 'Tenah' were used. With this homogeneous plant material and standardized cultivating methods, no effect of rearing conditions on the results of experiments would be expected.

Stock cultures were kept in a greenhouse at 20 ± 2 °C, 18L:6D, relative humidity 70-80%. Presexuals and males were produced and tested with short-days (10L:14D).

They were induced by transferring wingless females from long to short days. The second generation of host-alternating species, when reared under short day conditions, consists of gynoparae and males, while those of monoecious species consists of sexuparae (Blackman 1988).

Host plant choice was tested in a cage, 31cm x 22cm x 40cm, with dark walls and a fine gauze cover illuminated centrally from above. Only young, vigorous individuals were selected for the experiments. In each trial, two equal-sized plants of different species were placed in moist sand. The plants were vegetative, actively growing *R. nigrum* and *Lamium* species, but *R. rubrum* plants with mature leaves were also used. To test if the difference in leaf age between the *R. rubrum* and *R. nigrum* plants influenced host preference, experiments with *R. rubrum* plants bearing all young leaves (4-5 weeks after breaking of the buds) or all mature leaves (>8 weeks) were performed. Although *Ribes* plants with senescent leaves would be more appropriate, it was not possible to rear these in the greenhouse.

A choice experiment was started by releasing up to 120 aphids from a glass tube placed between the two plants. After 24 hours, the distribution of individuals and their offspring on each plant was counted (Rautapää 1970; Leather & Dixon 1982). Aphids found on the cage and the bottom were scored "no choice". Different morphs were tested separately and each experiment used at least two, but normally four replicates. On average 75% of the aphids were recovered in these experiments.

To determine host preference, the results of the choice experiments were summed for each clone and these were analysed using a X^2 test. Differences in preference between clones were analysed by calculating the proportion of aphids making a choice in each experiment (always more than ten individuals per test) and performing a one-way analysis of variance (ANOVA) on the arcsin-transformed values (Sokal & Rohlf 1981). Host plant suitability was measured in terms of aphid survival and offspring production by gynoparae over a period of 7 days.

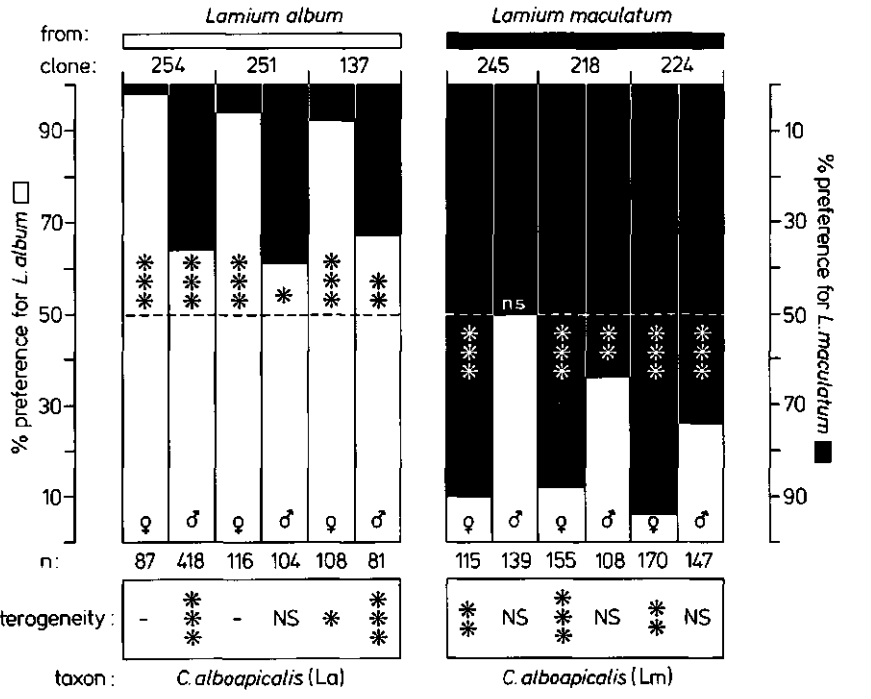

Figure 3.2 Preference shown by wingless sexuparae and males of clones of the monoecious *C. alboapicalis*(L. album) and *C. alboapicalis*(L.maculatum) for the host plants *Lamium album* and *L. maculatum*.
n = the number of recaptured individuals. No choice 6 (s.d. 7%, range 0-24%). X^2 test significance levels are indicated as * $P<0.05$, ** $P<0.01$; *** $P<0.001$; NS $P>0.05$. - = X^2 heterogeneity could not be calculated.

RESULTS

The presexual morphs and males of various forms of the three *Cryptomyzus* species showed marked differences in host plant preference. Those of the two monoecious forms of *C. alboapicalis*, given a choice between *Lamium album* and *L. maculatum*, show a marked preference for their field host (Figure 3.2). The preference shown by the wingless sexuparae was more pronounced than that of the males: 95% of the females of *C. alboapicalis*(L. album) chose *L. album*, compared with 61% of the (always wingless) males (ANOVA, $F_{[1,25]} = 16.023$, $P<0.001$). Similarly, 91% of the sexuparae of *C. al-*

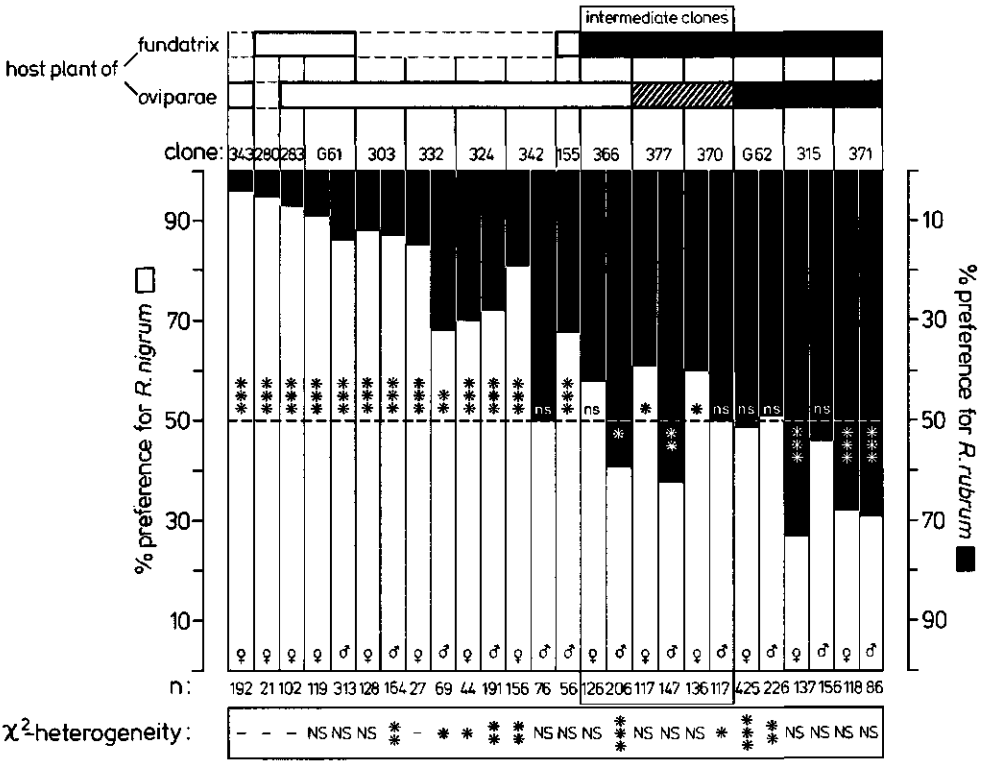

Figure 3.3 Preference shown by gynoparae and males of clones of the host-alternating *C. galeopsidis*(G.tetrahit) for the primary host plants *Ribes nigrum* and *R. rubrum*. Primary host of the fundatrix (stem mother) of the clones and hosts on which their oviparae matured are indicated on the top of the figure; hatched area indicate that oviparae matured on both *R. rubrum* and *R. nigrum*; broken lines indicate missing data.
n = the number of recaptured individuals. No choice 13 (s.d. 10%, range 3-46%). X^2 test significance levels are indicated as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS $P>0.05$. - = X^2 heterogeneity could not be calculated.

boapicalis(L. maculatum) selected *L. maculatum*, compared with 63% of the (always winged) males (ANOVA, $F_{[1,16]} = 16.228$, $P<0.01$). This resulted in only a weak difference between males of the two forms (ANOVA, $F_{[1,26]} = 2.607$, $P = 0.0536$).

When the *R. nigrum* and *R. rubrum* forms of *C. galeopsidis*(G. tetrahit) where offered a choice between the two currant species, the gynoparae and males of the *R. nigrum* form preferred *R. nigrum* and their oviparae matured on it. There was no differ-

ence in preference between males and females (ANOVA, $F_{[1,29]} = 1.652$, not signifi-cant). So host preference of the migrants was correlated with the suitability of that host for the oviparae produced (Figure 3.3). However individuals originated from *R. rubrum* were more variable in their host preference then those from *R. nigrum*. Two groups can be distinguished: (1) clones whose oviparae only matured on *R. rubrum* and whose gynoparae and males did not show a different host preference (ANOVA, $F_{[1,40]} = 0.210$, NS); (2) an intermediate group of clones whose oviparae matured on both *Ribes* species or only on *R. nigrum* (Figure 3.3). In this case the preference of gynoparae and males was different (ANOVA, $F_{[1,27]} = 4.442$, $P<0.05$). For the *R. rubrum* form, host plant choice was not always correlated with survival of the oviparae. Overall, the three groups (the *R. nigrum* form and the two groups of the *R. rubrum* form) differed in their preference for *R. nigrum* (ANOVA, $F_{[2,110]} = 57.508$, $P<0.001$). The females from each group showed different preferences (multiple range analysis with 95% confidence intervals), whereas only the males of the *R. nigrum* form differed from the other two groups.

To analyse the consequences of this variability, the fecundity of the gynoparae of several clones originated from *R. rubrum* was measured. The highest fecundity was recorded for those clones of which the oviparae developed only on one of the two currant species (Table 3.1). If oviparae of a clone matured on both *Ribes* species (Clones 370 and 377, Figure 3.3) their gynoparae performed better on *R. nigrum*, but on both hosts their fecundity was lower than the clones that produced optimally on those plants (Table 3.1). This suggests that there is a cost in terms of fecundity to being able to live on

Table 3.1 Survival (S) and mean seven day-fecundity (P7/♀) of the gynoparae of clones of *C. galeopsidis*(G. tetrahit) originated from *Ribes rubrum* and tested on *R. nigrum* and *R. rubrum*. exp = number of experiments, N = number of individuals tested. Different letters indicate differences at $P<0.05$, Mann-Whitney *U* test.

clone	N	S	P7/♀ ± sd	exp oviparae mature/not mature	N	S	P7/♀ ± sd	exp oviparae mature/not mature
	on *R. nigrum*				on *R. rubrum*			
371	40	.15	1.1 ± 1.0 a	?/?	50	.38	6.7 ± 3.5 c	5/0
315	34	.03	0 a	0/3	30	.37	4.8 ± 3.5 c	3/0
377	30	.13	3.5 ± 2.2 b	3/0	30	.03	0.4 ± 0.7 a	1/2
370	67	.48	3.6 ± 1.2 b	7/0	67	.01	0.2 ± 0.3 a	1/6
366	45	.53	7.8 ± 3.0 c	5/0	51	.11	0.0 ± 0.4 a	0/6

Table 3.2 Host plant preference shown by gynoparae (♀) and males (♂) of *C. galeopsidis*(G. tetrahit), clone G62, for *Ribes nigrum* plants with young leaves and *R. rubrum* plants with young (y) or mature (m) leaves, cultivars 'Fay Prolific' and 'Rovada'. Significance levels indicated as in Figure 3.2.

		Ribes nigrum	*Ribes rubrum*		no choice	$X^2_{(1)}$
			Fay Prolific	Rovada		
a	♀	210	215(m)		53	0.06 NS
	♂	115	111(m)		6	0.07 NS
b	♂	124	138(m)	-	33	0.75 NS
	♂	148	-	126(m)	31	1.77 NS
c	♀	40	104(y)	-	22	28.44***
	♀	32	-	113(y)	10	45.25***
d	♀	-	100(m)	52(m)	26	15.16***

both *Ribes* species.

Choice experiments with one clone that originated from *R. rubrum* indicated that the age of the leaves of a red-currant plant influenced the aphids' host choice. *R. rubrum* plants with young leaves were preferred to those of *R. nigrum* (Table 3.2c), but plants with mature leaves were not (Table 3.2a & b). Although the *R. rubrum* cultivar 'Fay Prolific' was preferred over the cultivar 'Rovada' (Table 3.2d), equal numbers of aphids were found on both cultivars when tested separately against *R. nigrum* (for males, Table 3.2b, $X^2_{(1)}$ = 2.52 NS; for females, Table 3.2c, $X^2_{(1)}$ = 1.25 NS).

Table 3.3 Host plant preference shown by gynoparae (♀) and males (♂) of a clone of *C. galeopsidis*(L. galeobdolon) and of *C. ribis*. Significance levels indicated as in Figure 3.2.

	R. nigrum	*R. rubrum*	no choice	$X^2_{(1)}$
C. galeopsidis(L. galeobdolon)				
♀	79	109	21	4.79 *
♂	40	44	13	0.19 NS
C.ribis				
♀	111	29	40	45.49 ***
♂	53	26	9	9.23 **

Gynoparae of *C. galeopsidis*(L. galeobdolon) showed a weak preference for *R. rubrum*, on which their oviparae matured, whereas males showed no preference (Table 3.3). *C. ribis* preferred *R. nigrum* (Table 3.3), while its oviparae matured on both *Ribes* species. For both aphid species, it seems probable that the preference for *R. rubrum* is underestimated, because *R. rubrum* plants with mature leaves were used. A careful interpretation of the results on preference is necessary if preference was not unambiguous and if results were heterogeneous within experiments (Figures 3.2 & 3.3).

DISCUSSION

What is the significance of host plant choice for reproductive isolation? Diehl & Bush (1984) gave many examples of closely related forms (host races), that differed in host preference and reproductive performance, so that there would be a decreased gene flow between the forms. Several species of aphids have such host-specific forms.

The aphid *Aphis fabae* Scopoli consists of several distinct forms (subspecies; Müller 1982), which all have one unique secondary host, though sharing many hosts (Iglisch 1968; Thieme 1988). Müller (1958) found differences in host preference of gynoparae and several forms also had a better performance on one of the primary host plants (Iglisch 1972). However, it remains obscure whether hybridization, which has been recorded under laboratory conditions (Müller 1982), occurs in the field.

In the present study, the mechanism of preference was not investigated. Only the final distribution of aphids on the different plant species was recorded. It may have been a response to visual, olfactory, tactile or nutritional cues. Repeated flights have a positive effect on the settling response of winged aphids (Johnson 1958). In this study the aphids were not subjected to a flight period before they were released in the test cage. Therefore it is possible that the proportion of colonization is suppressed. It was frequently observed that, after release, aphids initially flew to the cover of the cage, but settled on the hosts after a while. Wingless females have a stronger tendency to settle after a visit to a non-host (Klingauf 1976). This may increase the colonization of the preferred host in the experiments, since two different hosts were offered.

Host plant preference of the sexuparae and males of the two monoecious forms of *Cryptomyzus alboapicalis* from *Lamium album* and *L. maculatum* was different. Moreover, the two forms can only survive on their own host plant (Chapter 2). Because mating takes place on the host plant, the likelihood of cross-matings is reduced. However the two host plants sometimes grow side by side and dispersing males may encounter oviparous females of the other form. It is unknown whether hybridization occurs and whether it leads to viable offspring. Nevertheless, electrophoretic data indicate a complete separation of the two *C. alboapicalis* forms (Guldemond & Eggers-Schumacher 1989), which therefore should be considered as different species.

The host plant relationships of the *C. galeopsidis*(G. tetrahit) forms are complex. Hille Ris Lambers (1953) described two non-alternating subspecies, one on *R. rubrum* and one on *R. nigrum* (Figure 3.1). It was demonstrated experimentally that these forms cannot survive on each other's hosts (Guldemond 1987). Further, electrophoretic data indicated limited gene flow between these two forms (Guldemond & Eggers-Schumacher 1989). In the present study, the host preferences of the host-alternating forms were examined. If the gynoparae and males exclusively return to the species of primary host plant on which their fundatrices were born, these two forms are reproductively isolated. This condition might be satisfied by one group of clones that preferred *R. nigrum* and the other that preferred *R. rubrum*. Preference was correlated with the suitability of these hosts for the development of the oviparae. In these two forms, selective host preference leads to assortative mating and there is a potential for further evolutionary divergence by natural selection. This differs from the situation in *C. ribis*, where oviparae mature on both *Ribes* species, thus giving no indication for the existence of separated forms.

However, some clones (Figure 3.3; Clones 366, 370, 377) were more variable. Although they originated from *R. rubrum*, their oviparae matured mainly on *R. nigrum*, and less often on *R. rubrum* (Table 3.1). This group possibly consists of hybrids between the two populations which are more strictly confined to *R. nigrum* and *R. rubrum*. They produced fewer (Table 3.1) and smaller oviparae (unpublished data) than the other groups. This might indicate a reduced fitness for these intermediates. Therefore, the two *C. galeopsidis*(G. tetrahit) forms on *R. rubrum* and *R. nigrum* seem to be an example of host races (Jaenicke 1981; Diehl & Bush 1984).

To some extent, the *C. galeopsidis*(L. galeobdolon) form is reproductively isolated from *C. galeopsidis*(G. tetrahit) on *R. nigrum*. Gynoparae of *C. galeopsidis*(L. galeobdolon) show a preference for *R. rubrum*, on which plant the oviparae survive exclusively. Electrophoretic evidence suggests that these forms are closely related sibling species (Guldemond & Eggers-Schumacher 1989). It is unknown what isolating factors operate on the shared primary host plant *R. rubrum*.

Host choice in aphids is known to be influenced by the physiological condition of the plant, e.g. age (Kennedy & Booth 1951). Cereal aphids prefer young to mature leaves of some species of grass but preference of the same aphid for other grass species can be just the reverse (Rautapää 1970).

For gynoparae of one clone of *C. galeopsidis*(G. tetrahit), *R. rubrum* plants with young leaves were preferred to plants with mature leaves relative to *R. nigrum*. This seems surprising, because gynoparae return to their primary host in autumn when the currants no longer have young leaves. However in autumn, the leaves are senescent and these leaves may be more favourable then the mature leaves used in the experiments. Because red-currant plants with mature leaves were used in the host plant preference experiments, the choice for *R. rubrum* shown by clones from red-currant was underestimated. This strengthens the case for the existence of genotypes adapted specifically to red-currant.

Little is known about host plant choice by male aphids. Because oviparae of aphids are usually wingless and sedentary, the males have to move to find them. In general, the host plant choice of males was similar to that of gynoparae. The males of both forms of *C. alboapicalis*, however, showed a less marked preference than the females. As this aphid species is non-alternating, the males are produced on the right host plant. Because dispersal is not likely in this phase, they are adapted to search for females but show little special adaptation in searching for host plants. This might explain the frequent occurrence of males on the non-host plant in choice experiments.

Males of host-alternating species first have to find their primary host plant before they can start searching for a female. Within the homogeneous host-alternating *C. galeopsidis*(G. tetrahit) (the *R. nigrum* form and the *R. rubrum* form with their oviparae only maturing on *R. rubrum*), the preference of the males for *R. nigrum* did not differ significantly from that of the gynoparae. In contrast, the host preference of the intermediate

clones from *R. rubrum* differed between the sexes. The preference of the males is more inclined towards their original host *R. rubrum*, whereas the females incline towards *R. nigrum* on which the oviparae they produced developed optimally (Table 3.1; Figure 3.3).

From this study host plant preference of presexual morphs (sexuparae, gynoparae) and males of aphids proves able to act as a partial premating isolation factor. Females prefer their field host plant and, when they arrive on a (primary) host plant of a closely related form, they usually do not survive on it. Additionally, males prefer their field host plant. However some individuals will settle on the host of a related form and thus increase the chances of cross-insemination. Whether this leads to successful mating and viable offspring depends on how closely related the two forms are.

Changes in host plant utilization and with assortative mating can lead to speciation, even under sympatric conditions (Maynard Smith 1966; Bush 1975; Müller 1985). The occurrence of sympatric speciation is difficult to prove, but, because of their cyclic parthenogenetic nature and restricted host plant relationships (Futuyma & Peterson 1985), aphids seem to be good candidates for sympatric speciation.

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REFERENCES

Blackman, R.L. (1988) Rearing and handling aphids. In: World crop pests. Aphids, their biology, natural enemies and control. volume 2B. (ed. by A.K. Minks, P. Harrewijn), pp. 59-68. Elsevier, Amsterdam.

Bush, G.L. (1975) Sympatric speciation in phytophagous parasitic insects. In: Evolutionary strategies of parasitic insects and mites. (ed. by P.W. Price), pp. 187-206. Plenum, New York.

Dixon, A.F.G. (1971) The life-cycle and host preferences of the bird cherry-oat aphid, *Rhopalosiphon padi* (L.), and their bearing on the theories of host alternation in aphids. Ann. appl. Biol. **68**, 134-147.

Dixon, A.F.G. (1985) Aphid ecology. Blackie, Glasgow, pp. 168.

Diehl, S.R. & Bush, G.L. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. **29**, 471-504.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp. Roy. Ent. Soc. London **6**, London.

Futuyma, D.J. & Peterson, S.C. (1985) Genetic variation in the use of resources by insects. Ann. Rev. Entomol. **30**, 217-238.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 96-99. SPB Academic Publishing, The Hague.

Guldemond, J.A. & Eggers-Schumacher, H.A. (1989) Biosystematics of the aphid genus *Cryptomyzus*: an electrophoretic analysis. Z. zool. Syst. Evolut.-forsch. **27**, 14-25.

Hille Ris Lambers, D. (1953) Contribution to a monograph of the Aphididae of Europe V. Temminckia **9**, 1-176.

Hille Ris Lambers, D. (1966) Polymorphism in Aphididae. Ann. Rev. Entomol. **11**, 47-78.

Iglisch, I. (1968) Über die Entstehung der Rassen der "Schwartzen Blattläuse" (*Aphis fabae* Scop. und verwante Arten), über ihre phytopathologische Bedeutung und über die Aussichten für erfolgversprechende Bekämpfungsmassnamen (Homoptera: Aphididae). Mitt. Biol. BundAnst. Ld- u. Forst., Berlin-Dahlem, **131**, 1-34.

Iglisch, I. (1972) Zum Verhalten der "Schwarzen Bladläusen" (Arten und Rassen der *Aphis fabae*-Gruppe sensu stricto) auf ihren Primärwitspflanzen im Lauf der Vegetationsperiode (Homoptera: Aphididae). Z. angew. Zool. **59**, 153-160.

Jaenicke, J. (1981) Criteria for ascertaining the existence of host races. Am. Nat. **117**, 830-834.

Jermy, T. (1987) The role of experience in the host selection of phytophagous insects. In: Perspectives in chemoreception and behavior. (ed. by R.F. Chapman, E.A. Barnays & J.G. Stoffolano, Jr.), pp.143-157, Springer-Verlag, New York.

Johnson, B. (1958) Factors affecting the locomotor and settling responses of alate aphids. Anim. Behav. **6**, 9-26.

Kennedy, J.S. & Booth, C.O. (1951) Host alternation in *Aphis fabae* Scop. I. Feeding preferences and fecundity in relation to the age and kind of leaves. Ann. appl. Biol. **38**, 25-64.

Kennedy, J.S., Booth, C.O., Kershaw, W.J.S. (1959a) Host finding by aphids in the field I. gynoparae of *Myzus persicae* (Sulzer). Ann. appl. Biol. **47**, 410-423.

Kennedy, J.S., Booth, C.O., Kershaw, W.J.S. (1959b) Host finding by aphids in the field II. *Aphis fabae* Scop. and *Brevicoryne brassicae* L.; with a re-appraisal of the role of host-finding behaviour in virus spread. Ann. appl. Biol. **47**, 424-444.

Klingauf, F. (1976) Die Bedeutung der "Stimmung" im Leben phytophager Insekten am Beispiel des Wirtzwahl-Verhaltens von Blattläusen. J. appl. Ent. **82**, 200-209.

Leather, S.R. & Dixon, A.F.G. (1982) Secondary host preference and reproductive activity of the bird cherry-oat aphid *Rhopalosiphum padi*. Ann. appl. Biol. **101**, 219-228.

Maynard Smith, J. (1966) Sympatric speciation. Am. Nat. **100**, 637-650.

Müller, F.P. (1958) Wirtswahlversuche mit Gynoparen von *Myzus persicae* (Sulz.) und von *Aphis fabae* Scop. Z. PflKrank. PflSchutz **65**, 405-411.

Müller, F.P. (1982) Das Problem *Aphis fabae*. J. appl. Ent. **94**, 432-446.

Müller, F.P. (1983) Untersuchungen über Blattläuse der Gruppe *Acyrthosiphon pelargonii* im Freiland-Insektarium. Z. angew. Zool. **70**, 351-367.

Müller, F.P. (1985) Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. Biol. Zbl. **104**, 225-237.

Müller, F.P. & Hubert-Dahl, M.L. (1979) Wirtswechsel, Generationsfolge und reproduktive Isolation von *Ovatus crataegarius* (Walker, 1850) und *O. insitus* (Walker, 1850) (Homoptera: Aphididae). Dt. Ent. Z., N.F. **26**, 241-253.

Rautapää, J. (1970) Preference of cereal aphids for various cereal varieties and species of Gramineae, Juncaceae and Cyperaceae. Ann. Agric. Fenn. **9**, 267-277.

Sokal, R.R. & Rohlf, F.J. (1981) Biometry. Freeman & Co., San Fransisco.

Thieme, T. (1988) Zur Biologie von *Aphis fabae mordwilkowi* Börner und Janisch, 1922 (Hom., Aphididae). J. appl. Ent. **105**, 510-515.

Thompson, J.N. (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. exp. appl. **47**, 3-14.

4 Hybridization studies on the aphid genus *Cryptomyzus*, with observations on the genetics of host plant preference, reproductive performance and host-alternation

ABSTRACT

The aphid species *Cryptomyzus galeopsidis* (Kaltenbach) includes several distinct forms which have different host plant relationships and life cycles (Figure 4.1). Hybridization experiments were conducted to elucidate the taxonomic status of these forms and to investigate the inheritance of host preference, reproductive performance and host-alternation. One of the forms appeared to be a distinct species because of hybrid inferiority. Other host-alternating and non-alternating forms are considered conspecific and represent two life cycle strategies. Reproductive performance is probably controlled polygenically, since hybrids show an intermediate performance. Host preference in hybrids showed some degree of dominance and seemed to be determined by only a few genes. Host-alternation is presumed to be inherited monofactorially. The implications for speciation are discussed.

key words: aphid, *Cryptomyzus*, hybridization, host plant preference, reproductive performance, genetics, host-alternation, biosystematics.

INTRODUCTION

Hybridization studies are appropriate to show the genetic separation of populations and are often used to clarify the taxonomic status of closely related forms. Although the ability to hybridize in the laboratory does not necessarily reflect natural hybridization in the field, the apparent inability to form hybrids, or a reduced fitness of the hybrids indicates a genetic incompatability of the taxa studied.

The hybridization experiments of Müller and co-workers have greatly contributed to a better understanding of post-reproductive isolation factors in aphids. Hybrid sterility, hybrid inferiority, F_2 breakdown and reduced host finding ability have been recorded (Müller 1985).

Because aphids complete their whole life cycle on a host plant, the host may be considered to be an important factor in establishing reproductive isolation between populations and subsequent speciation (Guldemond, in press). Host plant preference and reproductive performance (see Thompson 1988a) are characters which form the liaison between aphid and host. Host-alternation is typical of aphids, entailing the obligatory switch in spring from the primary ("winter") host to the secondary ("summer") host and vice versa in autumn (Dixon 1985). These three traits are important factors which determine the clonization of a new, previously unused host and such a host shift may initiate speciation (Bush 1975; Diehl & Bush 1984; Futuyma & Peterson 1985).

The first step is the development of a preference for a new host (Jermy 1987) and simultaneously the ability to grow on the new host (Bush & Diehl 1982; Futuyma 1983). Genetic correlation between these traits would facilitate the divergence of the new population (Thompson 1988a; Singer et al. 1988). Assortative mating and disruptive selection would further advance the process of speciation (Maynard Smith 1966; Tavormina 1982). The frequency of such host shifts is also determined by the genetic basis of host preference and reproductive performance. The fewer the genes for controling these traits, the greater the chance that a host shift occurs. Experimental studies of the genetic basis of host preference and reproductive performance are scarce (Gould 1983; Futuyma & Peterson 1985; Thompson 1988b), in particular for pest species, which often extend their host range to previously unsuitable crops. An example in the case of aphids is the appearance of the sorghum-preferring biotype of the greenbug, *Schizaphis graminum*

(Rondani) in North America in 1968 (Blackman & Eastop 1984).

Aphids constitute an ideal group for the study of the genetic basis of host preference and reproductive performance. Because they are cyclically parthenogenetic, many crosses can be conducted with a single genotype and tests performed on each hybrid clone. Wipperfürth & Mittler (1986) and Newton & Dixon (1987) have recently devised methods to hatch eggs successfully in the laboratory, which will facilitate future genetic research on aphids.

The original hosts of aphids are presumed to be woody plants and host-alternation is evolved after the origin of herbaceous plants (Heie 1967). Hypotheses for the evolution of host-alternation are an optimalization of the available host plants contrary to a

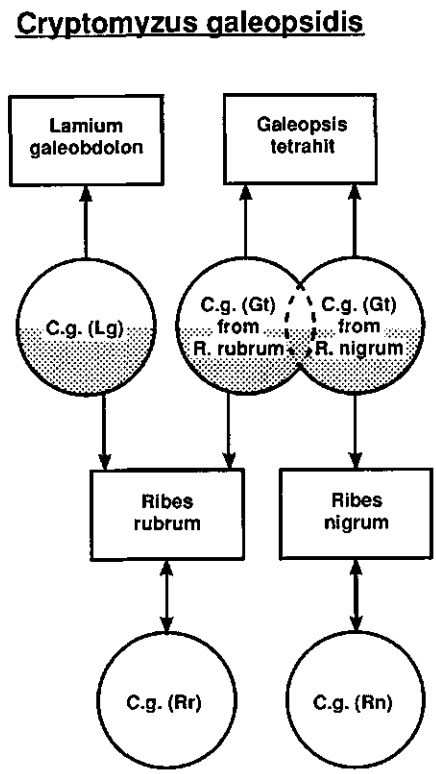

Figure 4.1 Host plant relationships and life cycles of the different forms of *C. galeopsidis*. Squares represent host plants and circles aphid forms, with the abbreviations of their respective host plants.

phylogenetic constraint which prevents the optimal use of the hosts (Moran 1988). The fact that more than 90% of the aphid species are non-alternating (Eastop 1973), combined with the theory of Hille Ris Lambers (1950) that often such species are derived from host-alternating ones, emphasizes the importance of host-alternation for speciation. So far, no studies have been performed on the genetic basis of host-alternation. Only Black-man (1981) studied the related topic of the inheritance of the holocyclic and anholocy-clic forms in *Myzus persicae* (Sulzer).

As part of a biosystematic study, the aphid *Cryptomyzus galeopsidis* (Kaltenbach) was studied. This species includes several forms which are host-alternating or just non-alternating. Different primary and secondary host plants are used and the taxonomic status of these forms is uncertain (Hille Ris Lambers 1953; Guldemond 1987).

The object of this study is to determine the degree of genetic divergence of the forms of *C. galeopsidis* by means of hybridization experiments. This will provide an indication of their taxonomic status. The hybrids were used to examine the inheritance of host plant preference, reproductive performance and host-alternation.

MATERIAL & METHODS

species studied

In *C. galeopsidis* the host plant relationships and life cycles of the different forms are indicated in Figure 4.1. On the primary ("winter") host plants *Ribes rubrum* L. and *R. nigrum* L. two forms are found, both of which host-alternate to the secondary ("summer") host *Galeopsis tetrahit* L.. These forms are referred to as *C. galeopsidis*(Rr/Gt) and *C. galeopsidis* (Rn/Gt), with the characteristic host plants in parentheses. Two non-alternating forms occur on the two *Ribes* species (Hille Ris Lambers 1953) and these are strictly host specific (Guldemond 1987). There is also a form that host-alternates between *R. rubrum* and *L. galeobdolon* (L.)L. referred to as *C. galeopsidis*(Rr/Lg) (Guldemond 1987) (Figure 4.1). The different clones of *C. galeopsidis* used in the experiments were all collected in the Netherlands within a range of one kilometer of each other. This implies that gene flow could have occured between the populations.

experiments

Rearing and experiments on the parthenogenetic morphs were performed under long-day conditions in a greenhouse (L18:D6, 20 ± 2 °C, r.h. 70-80%). Under similar but short-day (L10:D14) conditions the production and experiments on the (pre)sexual morphs (autumn migrants called gynoparae, which produce egg laying females called oviparae, and males) were carried out in a climate room. In the non-alternating forms the oviparae and males develop on the same host plant and thus larval oviparae had to be separated to obtain unmated females. In the host-alternating forms this was not necessary because only oviparae are produced by the gynoparae.

Experimental crosses were performed by confining oviparae and males on the same host plant (summarized in Table 4.1). In all crosses mentioned in the text the female parent is named first. After the eggs were laid in October-November, the cages were placed outdoors, because few eggs appeared to hatch under laboratory conditions.

In spring the females which hatched from the overwintering eggs (fundatrices) were reared singly or in groups on *Ribes* to obtain genetically independent cultures. The number of fundatrices used in the cultures is the maximum number of independent genotypes, because it is not known whether all fundatrices contributed equally to the production of offspring. The number of cultures represent the minimum number of different genotypes. Primary host plants of both parents were offered to the fundatrices. The development of these aphids on the primary host and the viability of their offspring

Table 4.1 The crosses between forms of *C. galeopsidis* performed in this study, with their characteristic primary/secondary host plants abbreviated, and their clone number. (Rr = *Ribes rubrum*, Rn = *R. nigrum*, Lg = *Lamium galeobdolon*, Gt = *Galeopsis tetrahit*). Crosses were performed on the primary host of the female.

female	*	male	female	*	male
forms with different secondary hosts			forms with/without host-alternation		
(Rr/Lg) 237	*	(Rr/Lg) 227	(Rr) G63	*	(Rr) G63
(Rr/Lg) 227	*	(Rr/Gt) G62	(Rr/Gt) G62	*	(Rr) G63
(Rr/Gt) G62	*	(Rr/Lg) 227	(Rr) G63	*	(Rr/Gt) G62
F_2 [(Rr/Lg 227 * (Rr/Gt) G62]			(Rr/Gt) G62	*	(Rr/Gt) G62
[(Rr/Lg) 227 * (Rr/Gt) G62] * (Rr/Lg) 227			(Rn/Gt) G61	*	(Rr/Gt) G62
			(Rn) 399	*	(Rn) 399 (field)
			(Rn) G60	*	(Rn/Gt) G61
			(Rn/Gt) G61	*	(Rn/Gt) G61

were recorded.

The experiments on host plant preference and reproductive performance were subsequently carried out with 1) spring migrants, 2) summer migrants (winged exules), which are the females that develop on and return to the secondary host plant, and 3) autumn migrants and males, which return to the primary host.

host plant selection

Host plant preference was measured in a cage using winged females, which were allowed to choose between two host plants. At least two experiments were performed on each clone. The experiments were carried out in a cage, 31 x 22 x 40 cm., with dark walls and a fine gauze cover, illuminated centrally from above. In each trial two plants of equal size of different species were placed in moist sand. In all choice experiments plants of similar age and size were used.

At the start of each experiment up to 120 aphids were released from a glass tube placed between the two plants. The different morphs were tested separately. After 24 hours the numbers of individuals and offspring on each plant were recorded (Rautapää 1970; Leather & Dixon 1982). Individuals found on the cage and on the ground were scored "no choice". At least 75% of the aphids were recovered in these experiments. For each clone the preference was assessed by pooling the data of the choice experiments, which were then analysed using a X^2-test. Differences in host preference between clones were analysed by calculating the percentage choice in each test (always more than ten individuals per test) and carrying out a one-way analysis of variance (ANOVA) on the arcsin-transformed values (Sokal & Rohlf 1981).

Host plant suitability was measured in terms of survival and reproduction of adult aphids on the test plant over a period of seven days. The reproduction of the hybrids was compared with that of the parents.

Host-alternating clones were defined as those whose spring migrants successfully reproduced on the secondary host plant *G. tetrahit*, but not on the primary host. Non-alternating genotypes demonstrated the reverse.

RESULTS

Hybrid viability

The forms of *C. galeopsidis* which live on the secondary hosts *L. galeobdolon* and *G. tetrahit* and which have *R. rubrum* as a primary host (Figure 4.2A), were found to hybridize under laboratory conditions. Of these Lg*Gt hybrids most cultures (13) reproduced normally on the primary host *R. rubrum*, while six others produced almost no winged spring migrants. The unwinged females were less viable and the cultures finally died out. In the reciprocal cross Gt*Lg 6 clones were studied of which only two produced a few spring migrants. These migrants produced very few offspring on the secondary hosts (Table 4.2) and none of them survived. Thus the colonization of a secondary host plant was not possible. Spring migrants of the only clone of the backcross (Lg*Gt)*Lg (Table 4.1) failed to establish themselves on either of the secondary host plants (n=15 on *L. galeobdolon* and n=14 on *G. tetrahit*).

The successful hybrids of the Lg*Gt cross survived on both secondary hosts, although their fecundity was lower than that of their parents (Table 4.3). Many of these hybrid spring migrants, compared with their parents, were unable to select a host and

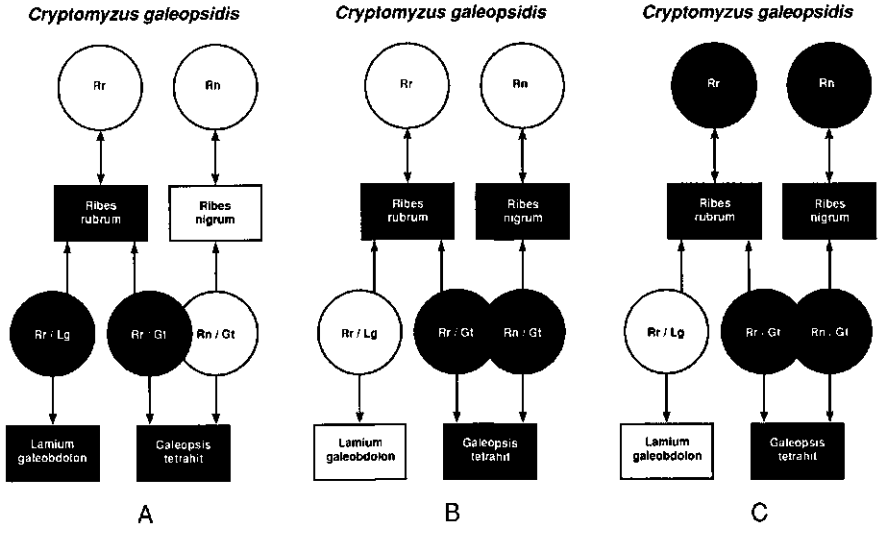

Figure 4.2 Host plant relationships and life cycles of forms used for the different hybridization experiments (in black).

Table 4.2 Mean 7 day fecundity of the spring migrants (P7/♀) of two forms of *C. galeopsidis* and their hybrids, which host-alternate between *Ribes rubrum* and the secondary hosts *Galeopsis tetrahit* (Gt) and *Lamium galeobdolon* (Lg) (Figure 4.2A).
(N = number of females tested, exp = number of experiments. The number of cultures and fundatrices used are: for Lg*Lg: 5 & 8; Lg*Gt: 7 & 20; Gt*Lg: 2 & 3. Values followed by a different letter differed at $P<0.05$, Mann-Whitney U).

SPRING MIGRANTS from *Ribes rubrum*

taxa in cross	tested on	P7/♀ ± sd	N	exp
(Lg) * (Lg)	*G. tetrahit*	0.06 ± 0.13 a	113	5
	L. galeobdolon	8.93 ± 3.70 c	43	5
(Lg) * (Gt)	*G. tetrahit*	3.79 ± 3.79 b	66	7
	L. galeobdolon	2.10 ± 1.83 b	85	7
(Gt) * (Lg)	*G. tetrahit*	0.38 ± 0.95 a	63	9
	L. galeobdolon	1.33 ± 1.30 b	62	9

settled on the cage or the ground (Figure 4.3; $X^2 = 42.18$, df = 1, $P<0.001$). Thus, although able to hybridize, the reduced fitness of the hybrids makes the establishment of permanent populations unlikely. This indicates that these two forms should be considered

Table 4.3 Mean 7 day fecundity of the summer migrants (P7/♀) of two forms of *C. galeopsidis* and their hybrids, which host-alternate between *Ribes rubrum* and the secondary hosts *Galeopsis tetrahit* (Gt) and *Lamium galeobdolon* (Lg) (Figure 4.2A).
(N = number of females tested, exp = number of experiments. Each taxon in cross consisted of one genotype, except Lg*Gt reared on *G. tetrahit* with two genotypes. Values followed by a different letter differed at $P<0.05$, Mann-Whitney U).

SUMMER MIGRANTS from secondary host plant

taxa in cross	reared on	tested on	P7/♀ ± sd	N	exp
(Lg)	*L. galeobdolon*	*G. tetrahit*	0.34 ± 0.52 a	107	9
	L. galeobdolon	*L. galeobdolon*	10.20 ± 3.90 cd	38	4
(Lg) * (Gt)	*G. tetrahit*	*G. tetrahit*	6.45 ± 3.90 bc	62	6
	G. tetrahit	*L. galeobdolon*	0.60 ± 1.10 a	96	6
	L. galeobdolon	*G. tetrahit*	5.29 ± 1.05 b	26	3
	L. galeobdolon	*L. galeobdolon*	0.27 ± 0.48 a	60	5
(Gt)	*G. tetrahit*	*G. tetrahit*	12.88 ± 3.03 d	61	6
	G. tetrahit	*L. galeobdolon*	0 ± 0 a	157	4

Table 4.4 Mean 7 day fecundity of the summer migrants (P7/♀) of two forms of *C. galeopsidis* and their hybrid, which host-alternate from *Ribes rubrum* (Rr/Gt) and *R. nigrum* (Rn/Gt) to the secondary host *Galeopsis tetrahit* (Figure 4.2B).
(N = number of females tested, exp = number of experiments. Each taxon in cross consisted of one genotype. Values followed by a different letter differed at $P<0.05$, Mann-Whitney *U*).

SUMMER MIGRANTS from and tested on *G. tetrahit*

taxa in cross	P7/♀ ± sd	N	exp
(Rn/Gt)	11.50 ± 4.25 a	30	3
(Rn/Gt) * (Rr/Gt)	12.70 ± 2.97 a	16	2
(Rr/Gt)	12.88 ± 3.03 a	61	6

as different species.

The cross between the two host-alternating forms from *R. nigrum* and *R. rubrum* (Rn/Gt*Rr/Gt), which share the same secondary host plant (Figure 4.2B), resulted in only one hybrid clone. The fundatrix fed on *R. nigrum* and produced few spring migrants, whose offspring thrived on the secondary host *G. tetrahit*. These summer migrants reproduced just as well on the secondary host as did their parents (Table 4.4). The gynoparae, on the other hand, produced oviparae on both *R. nigrum* and *R. rubrum*, but markedly fewer than their parents (Table 4.5), and frequently none of them matured. This indicates that the hybrid between the forms on *R. nigrum* and *R. rubrum* has a reduced fitness on the primary host plant. Furthermore, the number of hybrid gynoparae and males that had not made a choice was greater than of their parents (Figure 4.4; gynoparae $X^2 = 29.11$, males $X^2 = 25.98$, df = 1, $P<0.001$). This kind of hybrid inferiority had previously been described by Stroyan (1958) and Müller (1980, 1982). The taxonomic implications of these data are difficult to assess, because only one clone was studied.

Crosses between host-alternating and non-alternating forms of *C. galeopsidis* from *R. rubrum* (part of Figure 4.2C) revealed no hybrid inferiority. There are no reasons for treating these forms as different species.

Preference and performance of hybrids

Host plant preference of hybrids obtained by crossing the host-alternating forms of *C. galeopsidis* showed a (partial) dominance of one of the parental genotypes (Figures 4.3

Table 4.5 Mean 7 day fecundity of autumn migrants (gynoparae) (P7/♀) of two forms of *C. galeopsidis* and their hybrid, which host-alternate from *Galeopsis tetrahit* to the primary hosts *Ribes rubrum* (Rr/Gt) and *R. nigrum* (Rn/Gt) (Figure 4.2B).
(N = number of females tested, exp = number of experiments. Each taxon in cross consisted of one genotype. Values followed by a different letter differed at $P<0.05$, Mann-Whitney *U*).

AUTUMN MIGRANTS (gynoparae) from secondary host plant

taxa in cross	tested on	P7/♀ ± sd	N	exp
(Rn/Gt)	*R. rubrum*	0.03 ± 0.05 ab	40	4
	R. nigrum	9.35 ± 1.96 c	40	4
(Rn/Gt) * (Rr/Gt)	*R. rubrum*	0.40 ± 0.53 ab	26	3
	R. nigrum	1.37 ± 1.25 b	26	3
(Rr/Gt)	*R. rubrum*	5.40 ± 2.34 c	35	4
	R. nigrum	0 ± 0 a	36	4

& 4.4). Hybrid spring and summer migrants from the Lg*Gt cross (Figure 4.2A) both preferred *G. tetrahit* to *L. galeobdolon*, but spring migrants of the reciprocal cross, Gt*Lg, made no clear choice between the two secondary host plants, yet a small majority was found to have settled on *G. tetrahit* (Figure 4.3). This is in accordance with their larvae production in 24 h, which is greater on *G. tetrahit* than on *L. galeobdolon* ($X^2_{(1)}$ = 18.34, $P<0.001$). A significant difference in choice was found between the two crosses Lg*Gt and Gt*Lg (ANOVA, $F_{[13,1]}=4.718$, $P<0.05$).

Because the Lg*Gt cross gave the largest number of hybrid clones, their interclonal difference in preference could be assessed. The preference of Lg*Gt migrants for *G. tetrahit* ranged from 58 to 100%. Since even aphids from one-clone cultures may also display a wide variability in host preference, this does not necessarily indicate segregation for this trait (e.g. females of clone G62 showed 25-80% preference for *R. rubrum* in 13 experiments). The dominance in host preference exhibited by the hybrids possibly indicates genetic control of this character by a few genes, otherwise a more intermediate preference would have been observed. The use of the term dominance does not indicate that preference is inherited by alternative alleles.

The **reproductive performance** of hybrids was found to be intermediate between that of the parents (Tables 4.2, 4.3 & 4.5). The parents reproduced successfully on only one of the host plants, whereas the hybrids reproduced on both, although sometimes they

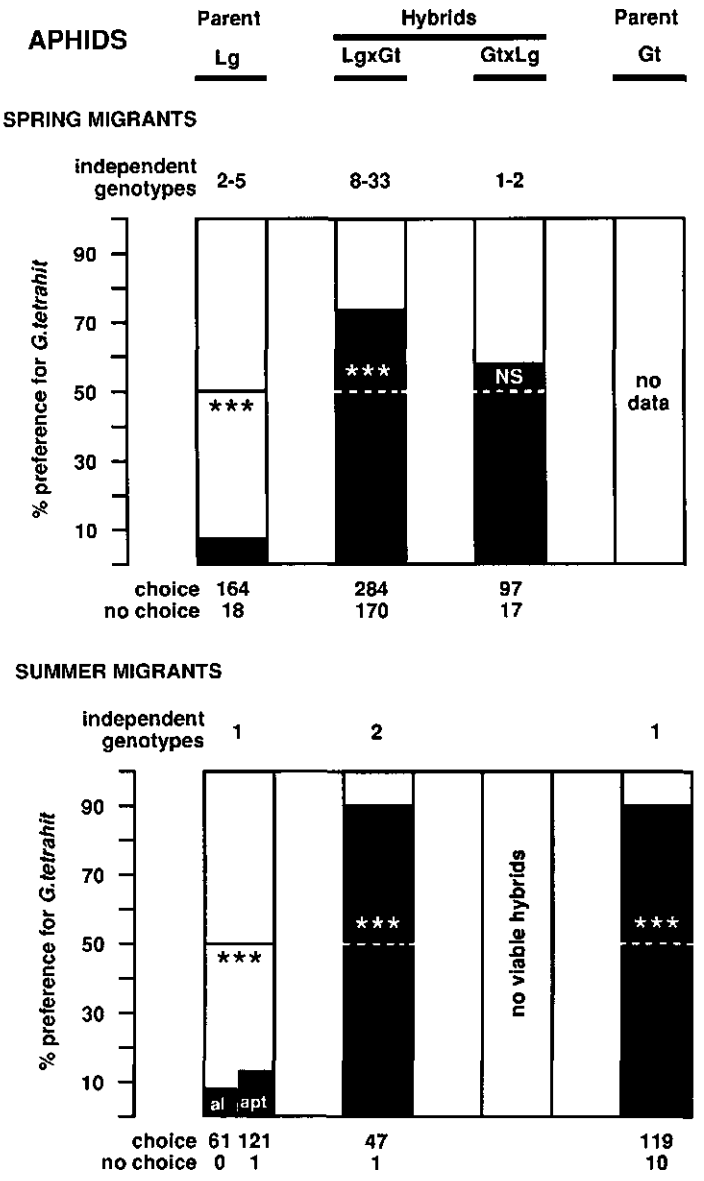

Figure 4.3 Preference of spring and summer migrants of *C. galeopsidis* forms, (Rr/Gt) and (Rr/Lg), and their hybrids, for the two secondary host plants *G. tetrahit* and *L. galeobdolon*. Parents of the summer migrants are reared on their natural host plant and the hybrid on both of the secondary hosts. The minimum number of independent genotypes is indicated by the number of cultures and the maximum by the number of fundatrices. Significance levels are ***P<0.001, NS P>0.05.

produced considerably less offspring.

In the case of the spring migrants of the Lg*Gt cross the different hybrid genotypes showed a wide range in fecundity on the two host plants (Figure 4.5). The extremes combined good performance on *G. tetrahit* with poor reproduction on *L. galeobdolon*, and vice versa. This indicates segregation of genetically determined performance factors in the F_1 generation implying that it is unlikely that performance is controlled by a few genes. There was no correlation between good performance on one host and a poor performance on the other (Spearman rank correlation coefficient, r_s=0.16, n=7, NS).

Gt*Lg spring migrants showed a low fecundity both on *G. tetrahit* and *L. galeobdolon* (Table 4.2), in contrast to females of the reciprocal cross, which reproduced as

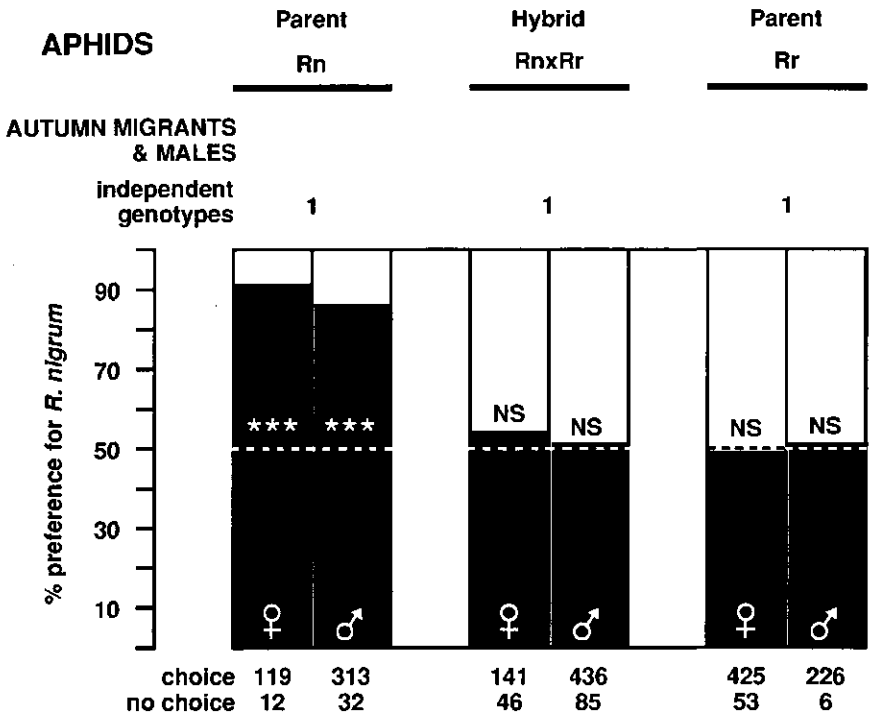

Figure 4.4 Preference of autumn migrants (gynoparae) and males of *C. galeopsidis* forms, (Rr/Gt) and (Rn/Gt), and their hybrids for the two primary host plants *R. rubrum* and *R. nigrum*. Significance levels are ***P<0.001, NS P>0.05.

well or even better on these hosts. However, because a wide range in performance may occur (Figure 4.5) and the fact that only two genotypes were tested, it is concievable that these genotypes represent extreme cases. The same explanation can be applied to the weak reproduction on *L. galeobdolon* of the summer migrants from the Lg*Gt cross (Table 4.3). Crosses between conspecific host-alternating and non-alternating genotypes (Figure 4.2C) showed that both traits were singly represented in the offspring, thus no clones showing both traits were found.

Host-alternation

A clone from *R. rubrum* appeared to be homozygous for non-alternation (Table 4.6[1]). Progeny of crosses between this clone and a host-alternating genotype showed

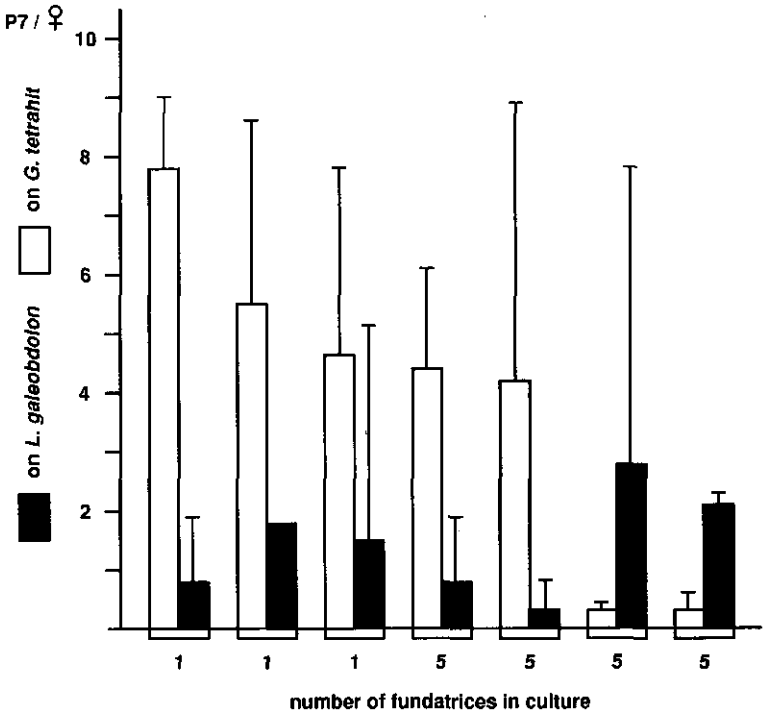

Figure 4.5 Mean 7 day fecundity of hybrid spring migrants (P7/♀) of the Lg*Gt cross. Seven genetically independent cultures were tested on *Galeopsis tetrahit* and *Lamium galeobdolon*. (two experiments with 5-25 females per culture/host plant).

Table 4.6 Number of clones with a host-alternating (HA) and non host-alternating (NHA) life cycle that resulted from crosses between HA and NHA forms of *C. galeopsidis* which use *R. rubrum* (Rr) and *R. nigrum* (Rn) as primary host plants (Figure 4.2C).

	female	*	male		Host Alternating	Non Host Alternating
crosses of non-alternating forms						
[1]	NHA (Rr)	*	NHA (Rr)		0	30
[2]	NHA (Rn)	*	NHA (Rn)	(field population)	2	1
crosses of alternating and non-alternating forms						
[3]	HA (Rr)	*	NHA (Rr)		7	4
[4]	NHA (Rr)	*	HA (Rr)		1	1
[5]	NHA (Rn)	*	HA (Rn)		0	2
crosses of alternating forms						
[6]	HA (Rn)	*	HA (Rr)		1	0
[7]	HA (Rr)	*	HA (Rr)		1	0
[8]	HA (Rn)	*	HA (Rn)		15	0

almost a 1:1 ratio (Table 4.6[3,4]; 8 host-alternating, 5 non-alternating clones), which indicates a simple Mendelian one-gene (or complex-gene) mechanism, with non-alternation as a recessive character. Consequently, the host-alternating genotype from *R. rubrum* should be heterozygous for this character, but this could not be determined because of the few numbers in the F1 generation. (Table 4.6[7]). Contrastly, clones from *R. nigrum* did not demonstrate that host-alternation was a recessive character. A host-alternating clone from *R. nigrum*, which appeared homozygous for this character (Table 4.6[8]), was hybridized with a non-alternating clone and this resulted in two non-alternating genotypes (Table 4.6[5]). A presumed non-alternating field population was collected on August 1, and consisted of 20 wingless females and larvae. After inbreeding both host-alternating and non-alternating clones were yielded (Table 4.6[2]), indicating that host-alternation is the recessive character. At this moment, it is not possible to arrive at an unequivocal conclusion regarding the genetics of host-alternation, although monofactorial inheritance is probable.

DISCUSSION

Taxonomy

The hybridization study revealed that the form of *C. galeopsidis* which migrates between *R. rubrum* and *L. galeobdolon* (Figure 4.2A) behaves as a distinct species. Evidence from an electrophoretic study supports this conclusion (Guldemond & Eggers-Schumacher 1989). The taxonomic status of the host-alternating forms on *R. rubrum* and *R. nigrum*, which migrate to *G. tetrahit*, is still uncertain (Figure 4.2B). The results of the hybridization experiments were not conclusive, but data on host plant preference indicate that intermediate genotypes between the more specialized *R. rubrum* and *R. nigrum* forms exist (Guldemond, in press).

The progeny of crosses between host-alternating and non-alternating clones did not show hybrid inferiority, and such crosses could occur in the field as well. This is corroborated by the non-alternating population on *R. nigrum* which after inbreeding yielded both host-alternating and non-alternating genotypes (Table 4.6[2])). Thus, host-alternation and non-alternation is seen as a dimorphic character within a population.

Host-alternation

The experiments demonstrated that host-alternation is probably controled by a single gene or a complex-gene, that is inherited monofactorially. In the first place hybrid clones with individuals which could reproduce on the primary as wel as on the secondary host were not found. Secondly, a non-alternating and host-alternating clone both showed no segregation in the F1, which makes polygenic control improbable (Table 4.6 [1,8]). The 'switching' of dominance, as the data are suggesting for host-alternation in clones from *Ribes rubrum* and *R. nigrum*, has been demonstrated for enzymes, and may be explained in this case by canalization between the relationship of gene activity and phenotype (Hoekstra et al. 1985).

The host-alternating gene may be regarded as a "switch-on switch-off" gene, which regulates the activity of a set of genes involved in preference and performance on either the primary or the secondary host. A similar regulatory system, based on a morphological study, had been reported by Akimoto (1985).

It may be advantageous for some aphid species to maintain two alternative life cycle strategies, as this would reduce competition and would spread the risk over two independent food resources. Adverse effects of weather on the survival of the migrants in spring and autumn could alter the ratio of the two forms from year to year. Moran & Whitham (1988) showed that ecological and genetic factors influence the ratio of two life cycles, a host-alternating and a reduced anholocyclic cycle on the secondary host, in populations of the aphid *Pemphigus betae* Doane.

The simple genetic control of host-alternation may have significant consequences for speciation. Suppose that one female which is heterozygous for host-alternation adapts to a previously unsuitable secondary host. By parthenogenetic reproduction during the summer a clonal population is built up. When the oviparae are produced on the new host, which is sometimes possible under adverse circumstances (Guldemond, unpublished results), sexual reproduction may take place. Then, recombination leads to a homozygous non-alternating population, which is "trapped" on the new host and a reproductively isolated population may evolve. Moreover, divergence to the species level is only a matter of time. This might be the way in which secondary monoecious (non-alternating) species have been evolved as described by Hille Ris Lambers (1950). Moran & Whitham (1988) consider life cycle reduction to be a gradual evolutionary process. It is unlikely that the reduction to an anholocyclic life cycle, as found in *P. betae*, leads to speciation. In many aphids those obligate parthenogenetic forms are found, but they normally remain closely related to the sexual form of the species (Blackman & Eastop 1984).

Preference & Performance

Host plant preference of *C. galeopsidis* is controlled by a small number of genes, as concluded from the dominance of one of the parental genotypes. For example, in the Lg*Gt cross, where at least eight genetically different hybrid populations (with 33 fundatrices) were studied, all populations preferred *G. tetrahit* (Figure 4.3). This was the case even for those clones that were more fecund on the other parental host plant, *L. galeobdolon*. When many genes control preference a more intermediate choice by the F_1 hybrids would have been expected.

Dominance in host plant preference in the F_1 generation has also been reported in the case of a cross between the so-called subspecies of *Acyrthosiphon pelargonii* (Kal-

tenbach) (Müller 1983). The F_2 hybrids segregated into two populations with different fecundities on, and preferences for, their parental host plants, which may indicate oligogenic control.

In other insect species the mode of inheritance of host preference may differ considerably. Evidence for monogenic control has been found in monophagous gall-forming flies (Huettel & Bush 1972), sawflies (Knerer & Atwood 1973), and planthoppers (den Bieman 1988). Oviposition preference in *Papilio* butterflies may result from as few as two loci or from interactions of more loci with a major X-chromosome effect (Thompson 1988c). Polygenic control of food preference, with some degree of dominance of one of the parental alleles, has been reported in *Drosophila tripunctata* (Jaenike 1985).

Reproductive performance in *C. galeopsidis* seems to be a polygenic trait, because the different F_1 clones of the Lg*Gt cross greatly differed in reproductive performance on their parental host plants (Figure 4.5). As a result the hybrids displayed various levels of fecundity in between that of the parents (Table 4.3). Similar experiments with other species of aphids gave rise to hybrids that either demonstrated intermediate reproductive performance between both parents, or exhibited dominance of one of the parental species. Sometimes both effects were found in one hybrid clone, e.g. the forms of the polyphagous *Aulacorthum solani* (Kaltenbach), which were tested on three different host plants (Müller 1976). Similarly, the larval survival of some species of sawflies was reported to be under polygenic control (Knerer & Atwood 1973). Virulence of the rice brown planthopper *Nilaparvata lugens* (Stål) on different rice varieties is consistent with polygenic determination (den Hollander & Pathak 1981), but in the aphid *Amphorophora ideai* (Börner) virulence on different resistent strains of raspberry, *Rubus idaeus*, is controlled by a single gene (Briggs 1965). In the biotypes of *Schizaphis graminum* the virulence of the aphid was inherited in an extra-nuclear manner from the mother. The transfer of symbionts was suggested as a mechanism (Eisenbach & Mittler 1987). In crosses with *Cryptomyzus* forms, no maternal effects were found for host preference or reproductive performance.

Due to the fact that the two forms of *C. galeopsidis* on *G. tetrahit* and on *L. galeobdolon* appear to be separate species, with their hybrids showing hybrid inferiority, the number of hybrid genotypes available for further study was limited. A viable F_2

could not be obtained, which has prevented a more detailed analysis of the genetical basis of preference and performance. In order to obtain sufficiently viable offspring crossings of forms that are more closely related would be required.

The mode of inheritance of host preference and reproductive performance may have consequences for speciation. When preference is determined by few genes, a new host plant can be colonized rather easily. If on the other hand performance is inherited polygenically, many mutations would be needed for a successful adaptation to a new host. This may explain why host shifts occur only rarely in nature.

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REFERENCES

Akimoto, S. (1985) Occurrence of abnormal phenotypes in a host-alternating aphid and their implications for genome organisation and evolution. Evol. Theor. 7, 179-194.

den Bieman, C.F.M. (1988) Hybridization studies in the planthopper genus *Ribautodelphax* (Homoptera, Delphacidae). Genetica **76**, 15-26.

Blackman, R.L. (1981) Species, sex and parthenogenesis in aphids. In: The evolving biosphere. (ed. by P.L. Forey), pp. 75-85. Cambridge University Press, Cambridge.

Blackman, R.L & Eastop, V.F. (1984) Aphids on the world's crops. John Wiley & Sons, Chichester.

Briggs, J.B., 1965. The distribution, abundance, and genetic relationships of four strains of the rubus aphid (*Amphorophora rubi* (Kalt.)) in relation to raspberry breeding. J. hort. Sci. **40**, 109-117.

Bush, G.L., 1975. Sympatric speciation in phytophagous parasitic insects. In: Evolutionary strategies of parasitic insects and mites. (ed. by P.W. Price), pp. 187-206. Plenum, New York.

Bush, G.L. & Diehl, S.R. (1982) Host shifts, genetic models of sympatric speciation and the origin of parasitic insect species. In: Proc. 5th Int. Symp. Insect-Plant Relationships. (ed. by J.H. Visser & A.K. Minks), pp. 297-306. Pudoc, Wageningen, Netherlands.

Diehl, S.R. & Bush, G.L. (1984) An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. **29**, 471-504.

Dixon, A.F.G. (1985) Aphid ecology. Blackie, Glasgow.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp. Roy. Ent. Soc. London **6**, London.

Eisenbach, J. & Mittler, T.E. (1987) Extra-nuclear inheritance in a sexually produced aphid: the ability to overcome host plant resistance by biotype hybrids of the greenbug, *Schizaphis graminum*. Experientia **43**, 332-334.

Futuyma, D.J. (1983) Selective factors in the evolution of host choice by phytophagous insects. In: Herbivorous insects: host seeking behaviour and mechanisms. (ed. by S. Ahmad), pp. 227-244. Academic Press, New York.

Futuyma, D.J. & Peterson, S.C. (1985) Genetic variation in the use of resources by insects. Ann. Rev. Entomol. **30**, 217-238.

Gould, F. (1983) Genetics of plant-herbivore systems. In: Variable plants and herbivores in natural and managed systems. (ed. by R.F. Denno & M.S. McClure), pp. 599-653. Academic Press, New York.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 96-99. SPB Academic Publishing, The Hague.

Guldemond, J.A. (in press) Choice of host plant as factor in reproductive isolation of the aphid genus *Cryptomyzus* (Homoptera, Aphididae). Ecol. Entomol.

Guldemond, J.A. & Eggers-Schumacher, H.A. (1989) Biosystematics of the aphid genus *Cryptomyzus* (Homoptera, Aphididae): an electrophoretic analysis. Z. zool. Syst. Evolut.-forschung, **27**, 14-25.

Heie, O.E. (1967) Studies on fossil aphids (Homoptera:Aphidoidea). Spolia Zool. Mus. Haun. **26**, 1-274.

Hille Ris Lambers, D. (1950) Host plants and aphid classification. Proc. 8th Int. Congr. Entomol., Stockholm, 141-144.

Hille Ris Lambers, D. (1953) Contribution to a monograph of the Aphididae of Europe V. Temminckia **9**, 1-176.

Hoekstra, R.F., Bijlsma, R. & Dolman, A.J. (1985) Polymorphism from environmental heterogeneity: models are only robust if the heterozygote is close in fitness to the favoured homozygote in each environment. Genet. Res., Camb. **45**, 299-314.

den Hollander, J. & Pathak, P.K. (1981) The genetics of the "biotypes" of the rice brown planthopper, *Nilaparvata lugens*. Entomol. exp. appl. **29**, 76-86.

Huettel, M.D. & Bush, G.L. (1972) The genetics of host selection and its bearing on sympatric speciation in *Procecidochares* (Diptera: Tephritidae). Entomol. exp. appl. **15**, 465-480.

Jaenike, J. (1985) Genetic and environmental determinants of food preference in *Drosophila tripunctata*. Evolution **39**, 362-369.

Jermy, T. (1987) The role of experience in the host selection of phytophagous insects. In: Perspectives in chemoreception and behavior. (ed. by R.F. Chapman, E.A. Barnays & J.G. Stoffolano, Jr.), pp. 143-157. Springer-Verlag, New York.

Knerer, G. & Atwood, C.E. (1973) Diprionid sawflies: polymorphism and speciation. Science **179**, 1090-1099.

Leather, S.R. & Dixon, A.F.G. (1982) Secondary host preference and reproductive activity of the bird cherry-oat aphid *Rhopalosiphum padi*. Ann. appl. Biol. **101**, 219-228.

Maynard Smith, J. (1966) Sympatric speciation. Am. Nat. **100**, 637-650.

Moran, N.A. (1988) The evolution of host-plant alternation in aphids: evidence for specialization as a dead end. Am. Nat. **132**, 681-706.

Moran, N.A. & Whitham, T.G. (1988) Evolutionary reduction of complex life cycles: loss of host-alternation in *Pemphigus* (Homoptera: Aphididae). Evolution **42**, 717-728.

Müller, F.P. (1976) Host and non-host in subspecies of *Aulacorthum solani* (Kaltenbach) and intraspecific hybridizations (Homoptera: Aphididae). Symp. Biol. Hung. **16**, 187-190.

Müller, F.P. (1980) Wirtspflanzen, Generationsfolge und reproductive Isolation intraspecifiker Formen von *Acyrthosiphon pisum*. Entomol. exp. appl. **28**, 145-157.

Müller, F.P. (1982) Das Problem *Aphis fabae*. J. appl. Ent. **94**, 432-446.

Müller, F.P. (1983) Untersuchungen über Blattläuse der Gruppe *Acyrthosiphon pelargonii* im Freiland-Insektarium. Z. angew. Zool. **70**, 351-367.

Müller, F.P. (1985) Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. Biol. Zbl. **104**, 225-237.

Newton, C. & Dixon, A.F.G. (1987) Methods of hatching the eggs and rearing the fundatrices of the English grain aphid, *Sitobion avenae*. Entomol. exp. appl. **45**, 277-282.

Rautapää, J. (1970) Preference of cereal aphids for various cereal varieties and species of Gramineae, Juncaceae and Cyperaceae. Ann. Agric. Fenn., **9**, 267-277.

Singer, M.G., Ng, D. & Thomas, C.D. (1988) Heritability of oviposition preference and its relationship to offspring performance within a single insect population. Evolution **42**, 977-985.

Sokal, R.R. & Rohlf, F.J. (1981) Biometry. Freeman & Co., San Fransisco.

Stroyan, H.L.G. (1958) Contribution to the taxonomy of some British species of *Sappaphis* Matsumura 1918 (Homoptera, Aphidoidea). J. Linn. Soc. **43**, 644-713.

Tavormina, S.J. (1982) Sympatric genetic divergence in the leaf-mining insect *Lyriomyza brassicae* (Diptera: Agromyzidae). Evolution **36**: 523-534.

Thompson, J.N. (1988a) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol. exp. appl. **47**, 3-14.

Thompson, J.N. (1988b) Coevolution and alternative hypotheses on insect/plant interactions. Ecology **69**, 893-895.

Thompson, J.N. (1988c) Evolutionary genetics of oviposition preference in swallowtail butterflies. Evolution **42**, 1223-1234.

Wipperfürth, T. & Mittler, T.E. (1986) A method for hatching the eggs of the aphid *Schizaphis graminum*. Entomol. exp. appl. **42**, 57-61.

5 Biosystematic study and morphometric discrimination of closely related aphid taxa in the *Cryptomyzus galeopsidis/alboapicalis* complex, with a key and revisionary notes on *Cryptomyzus* species of Europe

ABSTRACT

A morphometric study using canonical variates on closely related forms of the *Cryptomyzus galeopsidis* complex reveals dissimilarities between several forms characterized on the basis of their host plant relationships. A form which lives monoeciously on *Lamium maculatum* appears to typify *C. ulmeri* (Börner). Another taxon, morphologically very similar to *C. galeopsidis* (Kaltenbach), alternates between *Ribes rubrum* and *Lamium galeobdolon* and is described as *C. maudamanti* sp.n.. Linear discriminant functions have been derived to distinguish these taxa morphologically. The biology of the European *Cryptomyzus* species is described, including several previously unknown life cycles and morphs, and a key to wingless and winged virginoparous females is provided.

key words: aphid, *Cryptomyzus*, biosystematics, morphometry, host race.

INTRODUCTION

There is evidence that genetically isolated forms with different host plant relationships can exist within morphologically uniform groups of aphids (Müller 1985). These forms represent biologically different species, which are extremely difficult to identify. Even within the well studied polyphagous complex of *Myzus persicae*, new species have recently been described (Blackman & Paterson 1986; Blackman 1987). *Aphis fabae* Scopoli and *Acyrthosiphon pisum* (Harris) are other examples of complexes of species/forms which are morphologically difficult or even impossible to distinguish, and can only be characterized by their host plant relationships and life cycles (Müller 1971, 1982, 1985).

Similar phenomena occur in the genus *Cryptomyzus* Oestlund, which comprises several morphologically closely related but well defined species. Other species pose taxonomic problems e.g. the *C. galeopsidis* complex (Hille Ris Lambers 1953; Guldemond 1987; species names according to Eastop & Hille Ris Lambers 1976). Most *Cryptomyzus* species alternate between *Ribes*, which is the primary or "winter" host plant, and various species of Labiatae, which are the secondary or "summer" hosts. *C. galeopsidis* consists of different forms which are distinguished solely by their host plants and life cycle (Figure 5.1, see fold-out page at end of thesis). Host-alternating forms are found on *R. nigrum* L. and *R. rubrum* L., and the secondary host *Galeopsis tetrahit* L.. Hille Ris Lambers (1953) described non-alternating (monoecious) forms as subspecies: *C. g. dickeri* HRL from *R. nigrum* and *C. g. citrinus* HRL from *R. rubrum*. Recently, a form that alternates between *R. rubrum* and *Lamium galeobdolon* (L.)L., was identified (Guldemond, 1987). A closely related species, *C. alboapicalis* (Theobald), lives monoeciously on *L. album* L. and *L. maculatum* L. (Hille Ris Lambers 1953). When necessary the different forms mentioned in the text are followed by the abbreviation of their characteristic host plant(s) in parenthesis (see Figure 5.1).

Morphometric analyses, in combination with data on e.g. host plants and karyotype, can provide useful information on the taxonomic differentiation of closely related aphid forms and species (e.g. Blackman & Paterson 1986; Fargo et al. 1986; Brown & Blackman 1987). By using a canonical variate analysis, holocyclic and anholocyclic forms (Hand 1986) and insecticide resistant and susceptible individuals (Hampson & Madge 1986) have been separated.

The aim of this study is to determine whether there are morphometric grounds for

separating the different forms of *C. galeopsidis* and *C. alboapicalis*, which are currently distinguished by their host plants and life cycles, and to establish their taxonomic relationships. The biology of the other European species of *Cryptomyzus* is discussed briefly, unknown morphs are described, and a new key presented for the identification of wingless (apterous) and winged (alate) virginoparous females.

MATERIALS & METHODS

Material

For the morphometric study of wingless females, 36 samples collected in the Netherlands were studied seven of which were field samples and 29 laboratory reared clones. Each sample was assigned to one of the five putative taxa of *C. galeopsidis* or two of *C. alboapicalis* based on the reproductive performance of their winged females. To avoid circularity no morphological data were used to define these putative taxa (see Blackman & Paterson 1986). Field samples collected from a primary host in July and August were considered to be non-alternating taxa. Samples from *L. galeobdolon* (1) and *L. maculatum* (1) were designated as *C. galeopsidis*(Lg) and *C. alboapicalis*(Lm), respectively. For each taxon 25 to 61 individuals were measured, which gave a total number of 242 individuals. The characters were those used by Hille Ris Lambers (1953) to discriminate the various *Cryptomyzus* species. A preliminary morphometric study was also conducted with winged females, fundatrices (stem mothers) and oviparae. The characters and the number of individuals measured are listed in Tables 5.1 and 5.2.

Methods

A canonical variate analysis was conducted for the wingless females (SAS Institute 1982; Dunn & Everitt 1982; Blackman & Paterson 1986) and the population centres plotted. In discriminant analyses linear discriminant functions (LDF) are derived (Sneath & Sokal 1973), which designate an individual to a taxon, and the probability of belonging to that taxon calculated. For the winged females, fundatrices and oviparae the individuals were plotted onto the first two discriminant functions. Using a stepwise discriminant analysis (forward, backward, stepwise) the best discriminating characters were chosen and used to derive LDF's. For the wingless females the data were split into two

Table 5.1 Characters measured for the morphometric analysis and their abbreviations. Characters 1-16 for apterous, viviparous females; 3, 4, 6, 10, 15-20 for alate females; 1, 5, 18-21 for fundatrices and 1, 3, 4, 5, 18-20 for oviparous females.

Length of:

1. body, exclusive of cauda (body)
2. hind tibia (htibia)
3. processus terminalis of antennal segment VI (pt)
4. antennal segment III (antIII)
5. base of antennal segment VI (baseVI)
6. siphunculus (siphon)
7. longest hair on abdominal tergites II-IV (abhair)
8. longest hair on antennal segment I (hantI)
9. longest hair on antennal segment III (hantIII)
10. last rostral segment (lurs)
11. hind tarsus II (tarsII)

and:

12. maximal width of distal, swollen part of siphunculus (mawsi)
13. minimal width of proximal part of siphunculus (miwsi)
14. number of dorsal hairs on abdominal tergite III, except for very small ones (dhasIII)
15. number of additional hairs on last rostral segment, excluding the three pairs at the tip and two hairs at the base (hurs)
16. number of secondary rhinaria on antennal segment III (srhinIII)
17. number of secondary rhinaria on antennal segment IV (srhinIV)
18. length of antennal segment IV (antIV)
19. length of antennal segment V up to (including) primary rhinarium (antVa)
20. remaining part of antennal segment V (antVb)
21. diameter of base of antennal segment III (dbantIII)

equal sets, one of which was used to derive the LDF and the other to test this function (Blackman & Paterson 1986). Thereafter, the two sets of data were reversed and processed again and the most conservative estimate of the probabilities is given in the results.

Table 5.2 Number of samples examined, individuals and characters measured and the minimum and maximum number of individuals per taxon.

female morph	samples	individuals	characters	min-max ind/taxon
apterous	36	242	16	25-61
alate	19	87	10	11-17
oviparous	9	50	7	9-11
fundatrix	14	39	6	7-18

Subsets of the data were used in further analyses. Description of unknown morphs are based on about ten specimens.

In order to establish the host plant range of the species of *Cryptomyzus*, samples were collected in the Netherlands (201) and other European countries (46). Laboratory clones were reared in a greenhouse at 20 ± 2 °C, 70-80% relative humidity and L16:D8. Sexuals were produced at L10:D14. Chromosome preparations were made following Gut (1976).

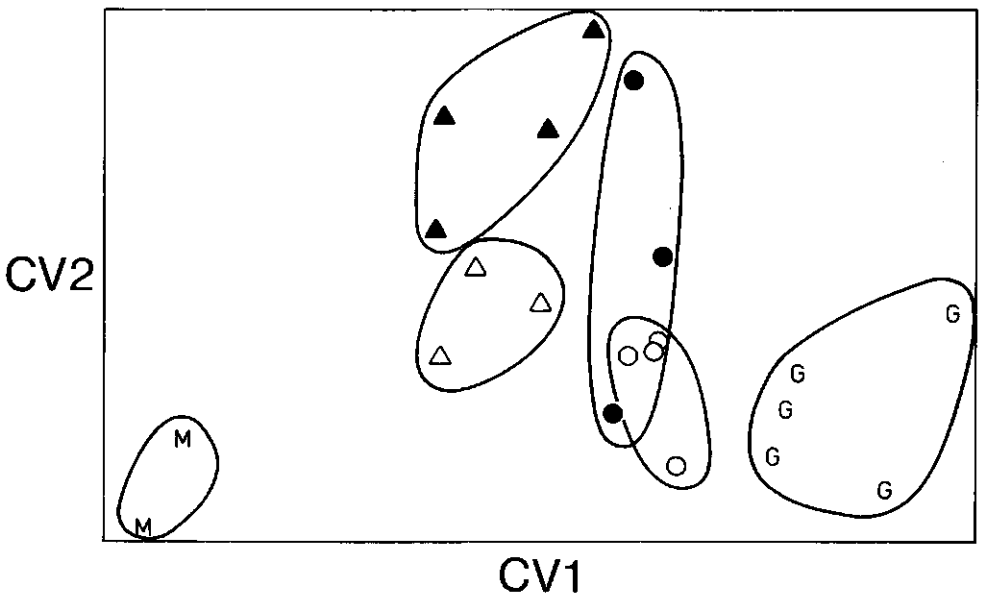

Figure 5.2 Plot of the scores on the first two canonical variates of the sample means of wingless females of the five forms of *C. galeopsidis* and one of *C. alboapicalis* (n=242).
M = *C. alboapicalis*(Lm), G = *C. galeopsidis*(Lg), o = *C. galeopsidis*(Rr),
● = *C. galeopsidis*(Rr/Gt), △ = *C. galeopsidis*(Rn), ▲ = *C. galeopsidis*(Rn/Gt).

RESULTS

Discriminant analysis

Figure 5.2 shows that most of the taxa in the *C. galeopsidis* species complex can be separated by plotting the first two canonical variates of the 21 samples of wingless females. *C. alboapicalis*(Lm) and *C. galeopsidis*(Lg) are both separated from *C. galeopsidis*, while the host-alternating and non-alternating forms of *C.galeopsidis* from *R. rubrum* overlap. Canonical variates 3 to 5 did not improve the separation of the forms. Morphological data seem to corroborate the data on host plants and life cycle in the differentiation between the taxa in the *C.galeopsidis* complex. When individual insects

Table 5.3 Classification results for wingless females using linear discriminant functions (in Table 5.4) based on all or the best four characters, performed on all data or on a test data set.

ACTUAL GROUP	PREDICTED GROUP							
	all (16) characters				best 4 characters			
	all data	test set			all data	test set		
	correctly classified	*albo*(Lm)	*gal*(Lg)+*gal*	correctly classified	correctly classified	*albo*(Lm)	*gal*(Lg)+*gal*	correctly classified
albo(Lm)	100%	12	0	99.2%	97.9%	12	0	96.7%
gal(Lg)+*gal*		1	107			4	104	
		gal(Lg)	*gal*			*gal*(Lg)	*gal*	
gal(Lg)	95.4%	29	2	93.5%	91.7%	28	3	88.9%
gal		5	72			9	68	
on *R. rubrum*								
gal(Lg)	98.5%				93.8%			
gal								
		gal(Rr/Gt)	*gal*(Rn/Gt)			*gal*(Rr/Gt)	*gal*(Rn/Gt)	
on *G. tetrahit*								
gal(Rr/Gt)	100%	14	1	78.1%	92.1%	9	5	83.9%
gal(Rn/Gt)		6	11			0	17	

Table 5.4 Linear discriminant functions (LDF's) to distinguish wingless *C. alboapicalis*(Lm) and *C. galeopsidis* forms. Measurements in mm and abbreviations as in Table 1.

1. 0.5(dhasIII) + 6976.2(tarsII) - 0.7(srhinIII) - 3089.1(miwsi) = 493.7
   C. alboapicalis(Lm) < 493.7 < *C. galeopsidis*(Lg) + *C. galeopsidis*

2. 1818.2(abhair) + 1130.3(bantVI) + 1.3(dhasIII) - 3275.5(hantI) = 200.2
   C. galeopsidis < 200.2 < *C. galeopsidis*(Lg)

3. 9746.5(miwsi) + 3443.3(abhair) - 1.3(dhasIII) - 2954.7(lurs) = 63.2
   On *G. tetrahit*: *C. galeopsidis*(Rn/Gt) < 63.2 < *C. galeopsidis*(Rr/Gt)

are plotted, instead of averages of samples, a considerable overlap between *C. galeopsidis*(Lg) and the *R. rubrum* form of *C. galeopsidis* was found.

For identification, a set of characters powerful enough to discriminate between the taxa is preferred; firstly, to distinguish the three most divergent forms morphologically, *C. alboapicalis*(Lm), *C. galeopsidis*(Lg) and *C. galeopsidis*, and secondly, to differentiate between the remaining forms of *C. galeopsidis*.

Linear discriminant functions

Table 5.3 shows the results of the classification of the different taxa based on the LDFs. When LDFs are calculated for the whole data set of the wingless females, including all 16 characters, 95% of the different individuals are classified correctly. Over 90% of the different individuals can be classified correctly using the best four discriminating characters, derived by stepwise discriminant analysis. With a test set, which consisted of one half of the entire data set, the derived LDF of the other half of the data set were tested. This resulted in a lower number of correctly classified individuals. The LDFs derived from the whole data set are presented in Table 5.4. A discriminant analysis for winged females of the different taxa revealed that the forms of *C. galeopsidis* are closely associated, and *C. alboapicalis*(Lm) and *C. galeopsidis*(Lg) can be separated (Figure 5.3). 94.2% of the individuals were classified correctly. Measurements of fundatrices of *C. galeopsidis*(Lg), and *C. galeopsidis* from *R. rubrum* and *R. nigrum*, were subjected to a

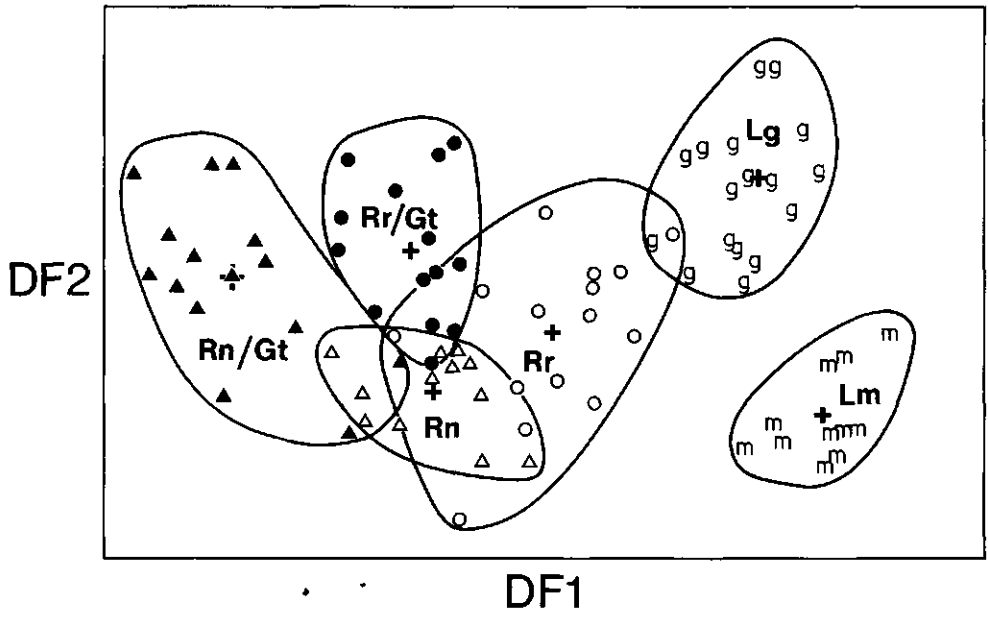

Figure 5.3 Plot of the scores on the first two discriminant functions of individual, winged females of the five forms of *C. galeopsidis* and one of *C. alboapicalis* (n=87). + = sample mean, otherwise the legend of taxa as in Figure 5.2.

discriminant analysis. Of the thirty-nine individuals from fourteen samples 33 (84.6%) were classified correctly.

C. alboapicalis(Lm) lives monoeciously on *Lamium maculatum*. When the host plant is known, *C. alboapicalis*(Lm) can only be confused with *C. galeopsidis*(Lg), because these are the only species of this complex capable of living on *L. maculatum* (chapter 2). Although other *C. galeopsidis* forms could not be reared on this plant, the possibility that some clones may survive cannot be excluded. Therefore, a LDF is useful to distinguish the different species with greater certainty. Using the LDF which includes all variables at least 99.2% of all individuals in the test set were classified correctly. With the best four variables separation was 96.7% (Tables 5.3 & 5.4).

Applying a LDF which includes all variables to the test set, 93.5% of the wingless females of *C. galeopsidis*(Lg) could be distinguished from those of other *C. galeopsidis*

forms. Using the best four variables 88.9% were distinguished (Tables 5.3 & 5.4). There may be confusion between the different forms particularly on the primary host *R. rubrum*. The LDF derived for individuals from this host alone is slightly more discriminating than the LDF for all individuals including those collected from other host plants (Table 5.3).

No characters were found which could singly separate the host-alternating from the non-alternating forms on *R. rubrum* or on *R. nigrum*. Of the wingless females of the two host-alternating *C. galeopsidis* forms, reared on the shared secondary host, *Galeopsis tetrahit*, 92.1% were correctly classified using a LDF based on the best four variables. Using test data at least 83.9% have been classified correctly (Tables 5.3 & 5.4).

The Mahalanobis distance (D) provides an indication of the morphological similarity of the taxa. This distance measured between the wingless females from the alternating and non-alternating taxa from *R. rubrum* is the smallest (D = 2.60), but the two taxa from *R. nigrum* are closer to those from *R. rubrum*, D = 3.85 and 3.79, than to each other (D = 4.51). This indicates that there is no unambiguous morphological evidence for the separation of *C. galeopsidis* taxa from *R. nigrum* and *R. rubrum* in the case of the wingless females.

Influence of host on morphology

Several taxa of the *C. galeopsidis* complex share *R. rubrum* as a primary host and this similarity may influence their morphology. Five groups were subjected to a discriminant analysis: *C. galeopsidis*(Lg) on *R. rubrum* and *L. galeobdolon*, the host-alternating *C. galeopsidis* on *R. rubrum* and secondary hosts and the non-alternating form on *R. rubrum*. It appeared that the taxa varied in morphology on the primary and secondary host, but the distinction between *C. galeopsidis*(Lg) on one hand and the taxa from *R. rubrum* on the other persisted (Figure 5.4).

DISCUSSION

Morphometry

Several factors may influence the morphology of an aphid and consequently the results of a morphometric analysis. Blackman & Paterson (1986) using a canonical variate anal-

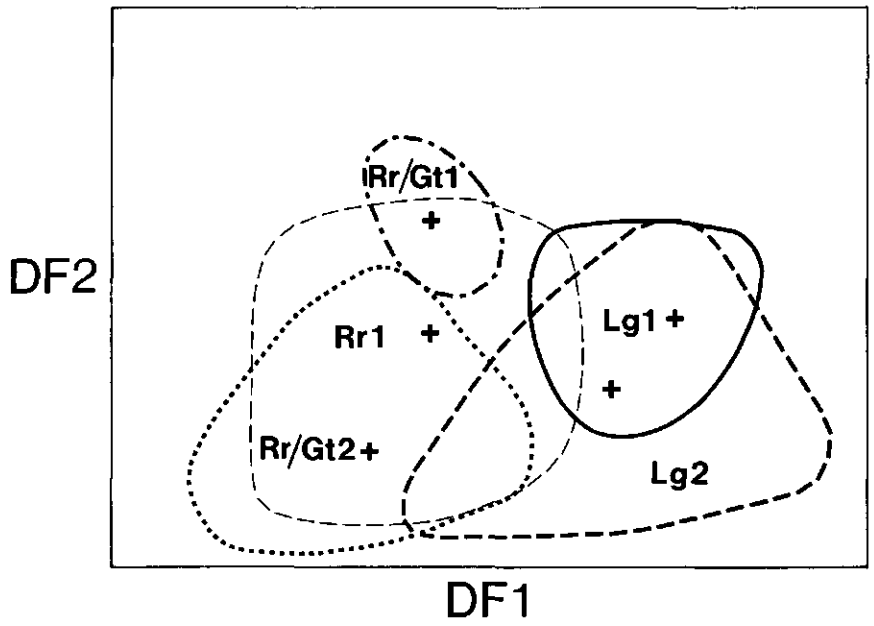

Figure 5.4 Plot of the scores on the first two discriminant functions of the (hand drawn) area occupied by wingless females of *C. galeopsidis*(Lg) and *C. galeopsidis*(Rn) and (Rn/Gt), which are collected on primary or secondary host.
1 = collected from primary host, 2 = collected from secondary host, + = mean of taxon*host.

ysis demonstrated the effect of rearing temperature on morphology. Nevertheless, aphids used in this study were reared at the same temperature, except for those collected in the field. A seasonal change in the length of the appendages was found in the sycamore aphid *Drepanosiphum platanoides* (Schrank) and in permanent parthenogenetic (anholocyclic) clones of the pea aphid *Acyrthosiphon pisum* (Harris) (Dixon 1974; Mackay et al. 1989). Genetic dissimilarities between host trees presumably influenced the morphology of *Pemphigus populitransversus* Riley (Bingham & Sokal 1986). To avoid biased samples, collections must be made throughout the entire season and from different hosts in order to determine the variability of the characters. Parasitized individuals, which could be included in field samples, may also differ in their morphology (Johnson 1959), yet in mounted specimens the presence of a parasite is usually detectable. In general, in order

to establish genetically determined disparities between taxa with a canonical variate analysis, the use of a data set of individuals reared under similar conditions from as many different clones as possible is sufficient. In contrast, to achieve a reliable LDF a wide range of characters should be measured, hence field samples and clones reared on different (primary and secondary) hosts must be included. The use of an independent data set is recommended for evaluating a LDF.

A canonical variate analysis can be applied in various ways to determine the morphological differentiation between a set of taxa. Individuals of one taxon can be dealt with depending on which taxon they belong to, or if a sufficient number of individuals per sample is measured, on the basis of the sample means, effected in this study for wingless females, following Blackman & Paterson (1986). First, the greatest distance of each individual of one sample is calculated in opposite of all other individuals of the other samples and so on for all samples. Secondly, the sample means are plotted and those of the same taxon are encircled by a hand drawn line. The results are derived therefore independently of the original classification of taxa. For other morphs of *Cryptomyzus* too few individuals per sample were measured to enable analysis of their relationships on the basis of sample means and so the individuals were grouped based on the taxon to which they belong using linear discriminant functions.

The number of linear discriminant functions derived is equal to the number of taxa analysed (SAS Institute 1982). When the five taxa of the *C. galeopsidis* complex and *C. alboapicalis*(Lm) are dealt with together, the values of six discriminant functions have to be calculated in order to allocate an unknown individual to a particular taxon. Because this is rather laborious the data set was divided into two parts for each case: one taxon to be separated as opposed to a combination of other taxa. Then, two linear discriminant functions are derived and by subtraction one function remains (Table 5.4).

The application of these discriminant functions showed that with all variables and the entire data set the taxa in Table 5.3 could be classified with a certainty of more than 95%. When the best four variables and a test set are used, only *C. alboapicalis*-(Lm) could be recognized with certainty. This indicates that this form of analysis has a limited value for identification and great care should be exercised using the discriminant functions.

TAXONOMY OF EUROPEAN *CRYPTOMYZUS*

Hille Ris Lambers (1953) provided an excellent survey of the genus *Cryptomyzus*. Recently, additional information has been supplied (Guldemond 1987), and the phylogenetic relationships based on allozyme data have been analysed (Guldemond & Eggers-Schumacher 1989). Börner (1930) erected a new subgenus *Myzella* in *Cryptomyzus* for *C. galeopsidis* and *C. alboapicalis* -whose close relationship is supported by allozyme data (Guldemond & Eggers-schumacher 1989)- and treated it as a separate genus in 1952 when adding a new species *M. ulmeri*. Eastop & Hille Ris Lambers (1976) considered *Myzella* to be a synonym of *Cryptomyzus* and *M. ulmeri* synonym with *C. alboapicalis*. They also placed *Amphorophora (Ampullosiphon) stachydis* Heikinheimo in *Cryptomyzus*.

In the following section the biology, morphology and distribution of the European *Cryptomyzus* species is described and discussed. Data on host plants are from chapter 2 and on allozymes from Guldemond & Eggers-Schumacher (1989, chapter 1). Because the means and ranges of morphological characters are required to enable the identification of several species and forms of *C. alboapicalis* and *C. galeopsidis*, data on the wingless and winged virginoparous females are provided in Tables 5.5 & 5.6. The geographical distribution is given in Table 5.7.

C. galeopsidis (Kaltenbach)

This species consists of two pairs of host-alternating and non-alternating forms, which are confined to *R. rubrum* and *R. nigrum*, and have *Galeopsis* as secondary host. The aphid causes the youngest leaves of *Galeopsis* to curl and leaf edges to roll (Docters van Leeuwen 1982; own observations). Other secondary hosts are *L. purpureum* L. and *L. amplexicaule* L., two plants on which all *Cryptomyzus* species may reproduce, and *Veronica agrestis* L. (chapter 2). Hille Ris Lambers (1953) found that sexuals of the monoecious form on *R. rubrum* appeared earlier, compared to the host-alternating form, and this may result in a reduced gene flow between these forms. Müller (1982) gives a similar example in *Acyrthosiphon pisum destructor* Johnson. Field experiments with caged monoecious forms showed that oviparae appeared at the end of August and males at the beginning of September and continued to produce sexuals right up to the appear-

ance of those of the host-alternating form in September/October. Some other monoecious clones only produced sexuals in this period. Field samples of the monoecious form from *R. nigrum* revealed that sexuals could be produced at the end of September. This indicates that some inbreeding of a monoecious form possibly occurs, but that sexual reproduction also may occur with the alternating form. No hybrid incompatability between forms from one primary host was found in laboratory crosses (chapter 4). The conclusion of Hille Ris Lambers (1953) that the non-alternating forms may be considered as subspecies of the alternating forms (*C. g. dickeri* on *R. nigrum* and *C. g. citrinus* on *R. rubrum*), is not supported by this study.

On the other hand, a division is detectable between the forms which are confined to *R. rubrum* and *R. nigrum*, respectively. These may be considered to be host races (definition follows Jaenike 1981), and hybridization might occur between them under natural circumstances (Guldemond, in press). It was demonstrated that the monoecious forms from *R. rubrum* and *R. nigrum* differed in allozyme frequency for the enzyme PGI (phosphoglucoisomerase), which may imply a reduced gene flow between these forms. Further, they could not survive on each other's host (Guldemond 1987). The gynoparae and males of the alternating forms preferred their original primary host on which also their oviparae matured, although some clones from *R. rubrum* showed a more intermediate behaviour (Guldemond, in press).

Morphologically, forms from *R. rubrum* and *R. nigrum* are difficult to differentiate, and are best characterized by their fundatrices: on *R. rubrum* these have relatively shorter abdominal hairs on segment II - IV. Maximal length of hair / diameter of the base of antennal segment III: 0.23 - 1.10 in *C. galeopsidis* from *R. rubrum*, 1.00 - 1.63 from *R. nigrum* and, in addition, 1.23 - 1.81 in *C. galeopsidis*(Lg) from *R. rubrum*. This distinction disappeared in the following generations. A character that may separate the two forms is colour: whitish-greenish for individuals from *R. nigrum* and more yellowish in the case of *R. rubrum* forms. Because of the existence of intermediates, whose fundatrices are born on *R. rubrum*, but whose oviparae develop optimally on *R. nigrum* (Guldemond, in press), this separation is not absolute.

Several small but significant differences between the forms of *C. galeopsidis* can be demonstrated, but these have little value for identification. Hille Ris Lambers (1953) discovered that the non-alternating form on *R. nigrum*, *C. galeopsidis dickeri*, had relatively longer hind legs and antennae. This was confirmed for wingless females: the hind

tibia / body ratio (one-way analysis of variance (ANOVA), $F_{[6,312]} = 21.944$, $P<0.001$) significantly differs from that of all other taxa in the *C. galeopsidis* complex, except for *C. galeopsidis*(Lg) (multiple range test); processus terminalis / body, $F_{[6,307]} = 45.465$, $P<0.001$, significantly different from that of all other forms. The relative distance from the distal end of the primary rhinarium on antennal segment V, compared to the total length of this segment, for winged females of *C. galeopsidis*(Rn) did not differ significantly from that of other taxa, as was stated by Hille Ris Lambers (1953). Additionally, contrary to his observations, the oviparae of *C. galeopsidis*(Rn) had swollen hind tibia as demonstrated in the other taxa, implying that this is not a useful character for discriminating between these taxa.

Differences between the forms of *C. galeopsidis* as described above, showed a more marked diversity between forms from *R. rubrum* and *R. nigrum*, than between host-alternating and non-alternating forms from the same primary host. Morphological data are given in Tables 5.5 & 5.6. From the non-alternating forms on *R. nigrum* and *R.rubrum* the chromosome number is 2n=12, reported by Blackman (1980) as well.

Distribution. Europe, from Finland to Spain and from Iceland (only on the primary hosts) and Ireland to Romania, Yugoslavia and Russia. The eastern limit however is unknown. Also USA and Canada, where they are probably introduced.

C. ulmeri (Börner) [=*C.alboapicalis*(Lm)]

Based on an extensive biosystematic study, it can be concluded that *C. alboapicalis*(Lm) is a distinct species. It lives monoeciously on *L. maculatum* and can easily be identified from *C. alboapicalis*(La), which lives on *L. album*, by the number of hairs on abdominal segment III of wingless females: 5-6 in *C. alboapicalis*(Lm) and 12-19 in the other species. The type specimen of *C. alboapicalis*, a winged female from *Malva*, has 14 abdominal hairs. Males of *C. alboapicalis*(La) are wingless (Hille Ris Lambers 1953; own data from 5 samples), while those of *C. alboapicalis*(Lm) are winged (data from 5 samples). Electrophoretic differences between the two species were found in the enzymes hexokinase (HK), 6-phosphogluconate dehydrogenase (6-PGDH), sorbitol dehydrogenase (SDH) and glycerol-3-phosphate dehydrogenase (GPDH).

Morphologically *C. alboapicalis*(Lm) resembles the taxa of the *C. galeopsidis* complex, but electrophoretically it differs in sorbitol dehydrogenase (SDH) and phosphoglucoisomerase (PGI). *C. galeopsidis*(Lg) was found to be the only other form of the complex that can reproduce on *L. maculatum*, although with a decreased fecundity. Sometimes, *C. alboapicalis*(Lm) can reproduce weakly on *L. album*.

Four wingless females of *C. ulmeri*, from *L. maculatum*, in Börner's collection were subjected to a discriminant analysis together with the other data. Their sample mean was situated closest to that of *C. alboapicalis*(Lm). This indicates that *C. alboapicalis*(Lm) resembles *C. ulmeri*, and therefore this is the proposed name for this taxon.

A description of the different morphs of *C. ulmeri* is given below and compared to data on *C. galeopsidis* (Kaltenbach) provided by Hille Ris Lambers (1953) and own data including *C. galeopsidis*(Lg). In Tables 5.5 & 5.6 morphological data on wingless and winged females are provided.

Apterous viviparous female. Rather similar to *C. galeopsidis* but smaller, 1.0 - 1.5 mm long, and (dark) green, with faint transverse bars on the back of the abdomen. On abdominal segments I-IV: on either side 1 - 2 marginal and 4 spino-pleural capitate hairs centrally. Only secondary rhinaria on antennal segment III: 0 - 7. The last rostral segment is rather blunt and short, 0.07 - 0.10 mm, with (2) 3 - 4 (5) additional hairs besides the three pairs at the tip. The siphunculi are short, 0.13 - 0.23 mm, with a slightly swollen distal part and a wrinkled base. The siphunculus / body length of *C. ulmeri* is shorter than in the *C. galeopsidis* forms. The (usually dark) green colour of *C. ulmeri* may distinguish it from the yellowness of *C. galeopsidis*(Lg), but pale individuals are also found. Other characters which are more or less distinctive between wingless females of these species are the length of the ultimate rostral segment, which ranges from 0.069 - 0.104 mm in *C. ulmeri* and from 0.106 - 0.129 mm in *C. galeopsidis*(Lg); the length of the siphunculus is 0.103 - 0.216 mm in *C. ulmeri* and 0.207 - 0.376 mm in *C. galeopsidis*(Lg).

Alate viviparous female. Resembles *C. galeopsidis*, but smaller, 1.5 - 1.9 mm long, and with a greenish abdomen and a dark brown sclerotized indented patch on tergites III-VI and stripes on I and II. Other parts mainly (dark) brown. Antennae longer than in *C. galeopsidis*: 1.6 - 1.7 times as long as the body. Secondary rhinaria on antennal segment

III: 15 - 28, on IV: 6 - 11 and on V: 0 - 5. Siphunculi more swollen than in apterous females, 0.18 - 0.21 mm long. Other characters as in apterous female. Winged females of *C. ulmeri* can be distinguished by a lower number of secondary rhinaria on antennal segment III: 18 - 25 (<4.4 / 0.1 mm), while other forms in *C. galeopsidis* have 25 or more (>5.1 / 0.1 mm).

Oviparous female. Much like apterous female, pale greenish, but with a more pointed distal part of the abdomen. Usually on either side one marginal and centrally four spinopleural hairs on abdominal segments I-IV. Secondary rhinaria on antennal segment III: 1 - 5. Siphunculi less swollen than in *C. galeopsidis* forms. The hind tibia are hardly swollen, with 40 - 74 pseudosensoria.

Alate male. Small, oblong body, light greenish. Transverse sclerotic bands on abdomen, which sometimes fuse to form a pale patch on tergites IV-VI. Secondary rhinaria on antennal segment III: 24 - 35, on IV: 10 - 19 and on V: 8 - 18. Otherwise like alate female.

Distribution. The material in Börner's collection came from Admont, Austria, and Naumburg, GDR. It has also been collected in the Netherlands, Belgium, FRG, Hungary and Czechoslovakia. Several of the records in the literature of *C. galeopsidis* from *L. maculatum* may belong to *C. ulmeri*.

Type. In his description of *Myzella ulmeri*, Börner (1952) did not designate a holotype. In the Börner collection in the Deutsches Entomologisches Institut, Eberswalde, GDR, there are three slides under *Myzella lamii* which apparently contain type material. I hereby designate the apterous viviparous female (nr 1) in slide 36/9, collected from *L. maculatum*, 8.VII.1938, Felsenkeller, Nbg (=Naumburg ?), GERMAN DEMOCRATIC REPUBLIC as the lectotype of *M. ulmeri*. I labelled it "*Myzella ulmeri*, lectotypes + paratypes, J.A. Guldemond, 1990". The two other slides I examined contain apterous females, larvae and winged males (slide 36/10, Admont, 30-07-1943 from *Lamium album* and slide 36/11, Nbg., 05-09-1940 from *Lamium amplexicaule*).

***C. maudamanti* sp. n. [=*C. galeopsidis*(Lg)]**

This taxon host-alternates between the primary host *R. rubrum* and the secondary host *L. galeobdolon*. Winged summer females prefer *L. galeobdolon* where its reproduction is optimal. They have reduced fecundity on the host of *C. galeopsidis*, *Galeopsis tetrahit*. No reproduction was possible on *Veronica agrestis* L., which is occasionally used by *C. galeopsidis*. Although not detected, the fact that a non-alternating form may exist on the primary host, as was found in *C. galeopsidis*, cannot be excluded. Allozyme data for the enzyme phosphoglucoisomerase (PGI) differ from that of other taxa of the *C. galeopsidis* complex. In hybridization experiments with another host-alternating *C. galeopsidis* form of *R. rubrum*, a reduced fecundity of the F_1 generations was found. On the basis of these data and morphological differentiation as shown above, it has been concluded that *C. galeopsidis*(Lg) is a separate species and is described here as *C. maudamanti*. There are no earlier names available for this species: examination of the original description of *C. galeopsidis* and of those previously used, invalid names (Hille Ris Lambers 1953), revealed that their data on morphology and host plants are inconsistent with *C. maudamanti*. Morphological data are provided in Tables 5.5 & 5.6. Chromosome number 2n=12.

Fundatrix. Seven fundatrices were examined from a cross between two clones of *C. maudamanti* reared in outdoor conditions. The fundatrices resembles those of *C. galeopsidis* from *R. nigrum*. The body is 2.0 - 2.7 mm, elongated, pale greenish-yellowish with a darker green spinal stripe. On either side one marginal and centrally, four capitate spino-pleural hairs on tergites I-IV, with the longest hair 0.049 - 0.068 mm. The antennae are shorter than the body, with a relatively long base of segment VI and a short distal end of segment V (including the primary rhinarium). The ratio distal end V / base VI is 0.31 - 0.44, compared with 0.34 - 0.63 in *C. galeopsidis*. Siphunculus length ranges from 0.47 - 0.55 (0.36 - 0.51 in *C. galeopsidis*), and each is slightly swollen distally.

Apterous viviparous female. Resembles *C. galeopsidis*. Oblong body, pale yellowish to light greenish, sometimes with a faint, green spinal stripe. Body is 1.1 - 2.3 mm long. On the tergites I-IV there are on either sides 2 (3 - 4) marginal and in the middle 4 - 6

(7) spino-pleural hairs (a total of 8 - 14 capitate hairs on strong bases), partly overlapping with the 4 - 11 hairs in *C. galeopsidis*. On tergite V there are 3 - 4 hairs. Antennae about 1.5 times the length of the body, with (0) 2 - 10 secondary rhinaria on the outer side of the base of segment III. The basal part of antennal segment VI is slightly longer and the siphunculi are more swollen than in *C. galeopsidis*. The rostrum usually reaches the third coxae and the last segment is more acute and longer but with a similar number of hairs (3 - 6) as *C. galeopsidis*. On the secondary host of *C. maudamanti*, *L. galeobdolon*, no other *Cryptomyzus* species survived (chapter 2), which makes it highly probable that wingless females collected from this host belong to *C. maudamanti*.

Alate viviparous female. Elongated, yellowish body (1.2 - 2.3 mm) with (light) brown legs and antennae. Abdominal markings as in *C. galeopsidis*. The same number of abdominal hairs as the apterous female, but much shorter and finer. Antennal segment III with 26 - 42 secondary rhinaria, IV with 4 - 22 and V with 0 - 9. Segment V is relatively longer than in *C. galeopsidis*. The rostrum is longer and reaches to at least halfway between the second and third coxae, and on the last segment there are 4 - 5 (6) additional hairs besides the three pairs at the tip. The siphunculi are swollen distally, have a wrinkled base and a flange, are a light brown colour and longer than in *C. galeopsidis*. Winged females of other forms of *C. galeopsidis* can incidently be found on other hosts. If they have more than 42 secondary rhinaria on antennal segment III they are unlikely to be *C. maudamanti*.

Oviparous female. Smaller than the apterous female, yellowish, with body tapering behind siphunculi. Fewer, and shorter, abdominal hairs than in apterous female, one marginal at either side of the tergites and four spino-pleural hairs centrally. The antennae lack secondary rhinaria. The rostrum reaches past the third coxae. The siphunculi are less swollen than in the apterous female. The hind tibia are slightly swollen, with 48 - 63 pseudosensoria. No differentiation with *C. galeopsidis* was found.

Alate male. Similar to winged female, but smaller. Antennae with 39 - 49 secondary rhinaria on segment III, 17 -28 on IV and 11 - 19 on V. Last rostral segment with 5 - 6 additional hairs besides the three pairs at the tip.

Distribution. *C. maudamanti* has been collected in the Netherlands, FRG and Czechoslovakia.

Type. The holotype is an apterous viviparous female reared in the laboratory on *Lamium galeobdolon* for one generation. The clone was collected on 21.VIII.1984, Hemmen, Gelderland, THE NETHERLANDS, on *L. galeobdolon*, slide nr. 06-6, population 205, individual nr. 5, the sample was taken on 25.IX.1984 and is in the collection at the Department of Entomology, Wageningen, the Netherlands. Paratypes are apterous and alate females and males from the same clone (Wageningen and British Museum (Natural History), London).

C. alboapicalis (Theobald)

This is a monoecious species that lives on *Lamium album*, can reproduce on *L. amplexicaule* and *L. purpureum*, but not on *L. maculatum*, the host of *C. ulmeri*. These two species exhibit the narrowest host range of all *Cryptomyzus* species. Despite the fact that *C. alboapicalis* was also reported on *Ballota nigra* L. (Hille Ris Lambers 1953), six clones did not accept this plant in laboratory experiments. A monoecious form of *C. alboapicalis* which lives on *B. nigra* may become reproductively isolated, as it does not survive on *L. album*. A laboratory experiment revealed that a clone from Calvados, France was anholocyclic. Allozyme data indicate that the closest relatives of *C. alboapicalis* are the species and forms of the *C. galeopsidis* complex. Morphological data in Table 5.5. Chromosome number 2n=12, as reported by Blackman (1980).

Distribution. Northern and Central Europe. From Norway to Finland and the European part of the USSR, from Poland, Switzerland to France and Great Britain.

C. leonuri Bozhko

This species lives on *Leonurus cardiaca* L. but no more details on the life cycle are known. Based on the original description and photographs (Bozhko 1961) it is con-

cluded that *C. leonuri* belongs to the group of species of *C. alboapicalis*, *C. galeopsidis*, *C. maudamanti* and *C. ulmeri*. The hairs on antennal segment III of wingless females are longer than the diameter of the base of segment III and about the same length as the hairs on segment I, the siphunculi are short and tiny, and the last rostral segment has few additional hairs in addition to the three pairs at the tip. The taxonomic position of *Leonurus* within the Labiatae is closer to *Galeopsis* and *Lamium*, the hosts of *C. galeopsidis* and *C. alboapicalis*, than to *Stachys*, the host of several other *Cryptomyzus* species (El-Gazzar & Watson 1970). Although this lends support to the suggested taxonomic relationship of *C. leonuri*, confirmation could be achieved by for instance electrophoretic data.

Distribution. Eastern Europe. Czechoslovakia, Poland and the European part of the USSR.

C. ribis (Linneus)

Probably the best known species of *Cryptomyzus*, because of the conspicuous red blisters it forms on *R. rubrum*. Other primary hosts are *R. nigrum* (pale blisters) and several other *Ribes* species (e.g. Keep & Briggs 1971). Sometimes chemical control is necessary because of a large production of honeydew. It host-alternates to *Stachys palustris* L. and occasionally to other *Stachys* species, *Leonurus*, *Galeopsis* and *Lamium*.

A separate form was found which remained on *R. rubrum* during the summer and produced wingless males (Hille Ris Lambers 1953). It is not known whether this is a truly monoecious form, because the preference of the winged females was not tested. I found that a host-alternating clone could be kept in a cage on *R. rubrum* during the whole season and then produced oviparae in October. Field populations of *C. ribis* were found on primary hosts untill the start of September, when they were usually killed by hymenopterous parasites, and syrphid and lacewing larvae. It remains to be proved that a monoecious form of *C. ribis* exists. An electrophoretic study revealed two alleles of the enzyme phosphoglucomutase (PGM) with only one heterozygous individual, which suggests the existence of two rather isolated forms. No correlation of these alleles with a particular host plant preference was found (Guldemond & Eggers-Schumacher 1989).

Chromosome number 2n=12, as reported by Robinson & Chen (1969).

Distribution. Europe, except Iceland and Portugal. Also Turkey, the Asian part of the USSR, Japan, Korea, North America and Mexico.

C. korschelti Börner

This species alternates between *Ribes alpinum* L. and *Stachys sylvatica* L.. The secondary hosts *Lamium purpureum*, *L. amplexicaule* and *Galeopsis* are also accepted. It is capable of persisting on the primary host until the middle of September. In the laboratory a clone kept on *R. alpinum* produced very small wingless males. This is similar to the situation in *C. ribis* (Hille Ris Lambers 1953), and this aptery is possibly induced by continuous rearing on the primary host. Wingless males were also produced on the secondary host under changing scotophases. Other experiments showed that a clone from Calvados, France, was anholocyclic. Allozyme data demonstrated a close relationship between *C. korschelti* and *C. ribis*.

Oviparous female. Smaller than the wingless female (Hille Ris Lambers 1953), with a tapering abdomen behind the siphunculi, and yellowish in colour. The hairs on abdominal tergites I-IV: on each side 1 - 3 marginal hairs, which usually includes one short hair, and centrally 2 - 4 spino-pleural hairs, long and knobbed with a strong base. The spinal hairs are usually the longest and the pleural hairs are sometimes absent. Antennae longer than the body, with no (exceptionally one) secondary rhinaria on segment III. The rostrum reaches the third coxae and its last segment bears 10 - 13 additional hairs besides the three pairs at the tip. Siphunculus as in apterous female, but less swollen. Hind tibia are swollen, occasionally brownish, and bear a variable number of 56 - 130 pseudosensoria.

Apterous male. Small, yellowish body, with sclerotized marginal and spino-pleural brownish patches on the abdominal segments, sometimes fused into transversal bands or a large spot on segments IV-VI, and a large post-siphuncular spot. On the abdominal tergites there are on each side 2 - 3 marginal and centrally 4 - 6 spino-pleural hairs.

The number of secondary rhinaria on the antennal segments is dependent on the host plant: males from *R. alpinum* have on segment III: 15 - 20, IV: 4 - 9, V: 3 - 9; those from *S. sylvatica* have on segment III: 34 - 49, IV: 15 - 24, V: 11 - 12. This difference is partly due to the reduced size of the males from *R. alpinum*. The long rostrum reaches past the third coxae. Further characters as in the winged males.

Distribution. Europe. From Finland to Spain and from Great Britain to Italy and Romania, and the European part of the USSR.

C. heinzei Hille Ris Lambers

This species was originally only found on *Satureja vulgaris* Fritsch. Thanks to H.A. Eggers-Schumacher it has been discovered on *Ribes alpinum*. Here, pale yellow-green blisters are formed on the young leaves, causing them to bend. These plants grew in shade so that the colour of the blisters may be different if exposed to the sun. *C. heinzei* can remain on the primary host at least until the beginning of July. Winged females were successfully transferred from the primary to the secondary host *Stachys officinalis* (L.) Trev., for which field records are known. In contrast, all attempts to rear this species on *Satureja* were unsuccessful and no other records of *C. heinzei* from this host are known. It was concluded that *Satureja* is an unusual host or a misidentification.

Because of morphological similarities, Hille Ris Lambers (1953) did not exclude *C. heinzei* as being a form of *C. korschelti* reared on an unsuitable host for instance. Allozyme data however, demonstrate clear differences between these species and a close relationship of *C. heinzei* to *C. ballotae*.

Alate viviparous female. Body 1.2 - 1.5 mm, yellow, with head, thorax and extremities brown (apterous viviparous females on the primary host pale yellowish and on the secondary host pale whitish to bright yellow). The distal end of the antennae pale, knees and apical part of tibia and tarsi dark brown and siphunculi light brown. Abdomen with a more or less rectangular, indented, brown patch on tergites III-VI, brown marginal sclerites and post-siphuncular patch. Abdominal patch similar to that of *C. korschelti*, but more indented and abdomen lacks the rows of brown patches on tergites I and II. Ab-

dominal hairs on segments I-IV: on each side 1 - 3 marginal and 4 - 8 spino-pleural hairs centrally. Antennae almost twice the length of the body and the processus terminalis about 9 times the length of the base of antennal segment VI. Secondary rhinaria on segment III: 30 - 46, IV: 14 - 20 and V: 1 - 7. The last rostral segment is acute, with 7 - 9 additional hairs besides the three pairs at the tip. Siphunculi with a flange, regulary shaped, with the distal part swollen, 1.5 - 2.0 times the narrowest width, wrinkled at the base.

Oviparous female. Similar to apterous female but much smaller, 0.8 - 1.1 mm, pale whitish. Abdominal tergites with on each side 1 - 2 marginal and 4 - 5 spino-pleural hairs centrally, which often differ in length. Antennae much longer than the body, and lack of secondary rhinaria. The last rostral segment has 7 - 9 additional hairs besides the three pairs at the tip, which is less pointed than in the apterous viviparae. Hind tibia not or only slightly swollen, with few (21 - 29) pseudosensoria on the proximal 2/3 rds.

Alate male. Body yellowish, like alate female but with brown stripes fused to form a patch on abdominal tergites III-VI. Chaetotaxy like the alate female, but sometimes there is a reduced number of hairs. Antennae almost twice the length of the body, with secondary rhinaria on antennal segment III: 44 - 61, IV: 19 - 29 and V: 11 - 17. The last rostral segment with 8 - 10 additional hairs besides the three pairs at the tip. Siphunculi like those in alate females, but sometimes narrower at the base.

Distribution. Central Europe. Hungary, Czechoslovakia, FRG, and also found in Spain in the Pyrenees at 1300 m.

C. ballotae Hille Ris Lambers

This species lives on *Ballota nigra* and also reproduces well on *Lamium album*, *L. purpureum* and *L. amplexicaule*, however less so on *L. maculatum*, *Galeopsis*, *Stachys annua* (L.)L., *S. officinalis* and *Leonurus*. *C. ballotae* exhibits together with *C. ribis* the greatest secondary host range. Although the species has not been found on any primary host in the field, laboratory experiments showed that oviparae could be produced on *R.*

alpinum. Populations seem to be anholocyclic when tested in the laboratory under short day conditions for two to three generations (Blackman 1988) and have been collected in England in the months December - February (British Museum (Natural History), London). Only after a long exposure to short day conditions in the laboratory do clones produce a very few gynoparae and males. Thus it seems unlikely that these clones produce their sexuals like normal holocyclic clones. Chromosome number 2n=12, as described by Blackman (1980).

Oviparous female. Smaller than the apterous female, 0.9 - 1.2 mm, pale whitish. The hairs on the abdominal segments I-IV: on either side 2 - 4 marginal and 6 - 11 spinopleural hairs centrally, capitate with a strong base and differing in length. Antennae about twice the length of the body, with usually no, but sometimes up to three secondary rhinaria on antennal segment III. The last rostral segment has 8 - 10 additional hairs besides the three at the tip. The siphunculi are more or less straight or swollen as in the apterous females. The hind tibia are only slightly thickened, with pseudosensoria on the proximal half except for the first 1/8 - 1/10 part.

Alate male. Small body, 1.1 - 1.4 mm, green, like the apterous and alate females, the colour is much more intense than in most other *Cryptomyzus* species. Head, thorax, antennae, rostrum are (dark) brown, legs brown but with the proximal part of the femur lighter in colour and siphunculi are pale brown. Irregular, indented sclerotized brown patch on abdominal tergites III-V, which bears several unsclerotized stripes and spots. Brown, marginal sclerites and a large, rectangular post-siphuncular spot. The hairs on the abdominal tergites are shorter, thinner and hardly capitate, and about half the number of those of apterous females. The antennae are much longer than the body with secondary rhinaria on segment III: 45 - 60, IV: 12 - 24 and V: 6 - 11. The last rostral segment is pointed with 8 - 11 additional hairs besides the three at the tip. Siphunculi swollen on the inner side distally, and with a flange at the distal end.

Distribution. From Western to Southeastern Europe, including Great Britain, the Netherlands, France, Czechoslovakia, Bulgaria, Cyprus, Italy and Spain.

***C. (Ampullosiphon) stachydis* (Heikinheimo)**

The biology of this species has been described by Stenseth (1971). The primary hosts are *R. rubrum* and *R. spicatum* Robson and secondary hosts used in the field are *Galeopsis bifida* Boenn., *Stachys sylvatica* and *Lamium album* (O. Heikinheimo, pers. comm.; Szelegiewicz 1968). This host range was confirmed in laboratory tests and *G. tetrahit*, *S. palustris* and *L. amplexicaule* were added. The aphid causes discoloration of the veins of the youngest leaves of *S. sylvatica* in the laboratory, which was not observed in the field (Heikinheimo 1955; Stenseth 1971). The relationship of *C. (A.) stachydis* to *Cryptomyzus* is not unambiguous, because several characters also indicate a relationship with *Nasonovia* Mordvilko and *Hyperomyzus* Börner (Stenseth 1971), both of which are closely related to *Cryptomyzus* (Guldemond & Eggers-Schumacher 1989). The allozyme evidence is not conclusive because these characters may be homoplaseous, but it indicates that this species forms the sister group of the other *Cryptomyzus* species (Guldemond & Eggers-schumacher 1989). Just as other *Cryptomyzus* species, *C. (A.) stachydis* possesses a filter-gut (pers. comm. B. Ponsen), which strongly implies a close relationship between these taxa. Chromosome number 2n=12.

Distribution. Northeastern Europe: Norway, Finland and Poland.

Table 5.5 apterous viviparous females. Morphometric data of *Cryptomyzus* species showing the number of measured specimen, mean ± s.d. and extreme values in parentheses for characters described in Table 5.1.

	C. alboapicalis	*C. ulmeri*	*C. galeopsidis*(Rr)	*C. galeopsidis*(Rr/Gt)
number	51-52	33-40	52-54	43-46
body	1.78 ± 0.26 (1.45-2.13)	1.24 ± 0.19 (0.84-1.54)	1.55 ± 0.33 (1.10-2.29)	1.77 ± 0.35 (0.96-2.14)
htibia	1.41 ± 0.10 (1.19-1.68)	0.93 ± 0.13 (0.70-1.14)	1.30 ± 0.19 (0.98-1.80)	1.34 ± 0.28 (0.77-1.90)
tarsII	0.102± 0.005 (0.085-0.115)	0.068 ± 0.004 (0.057-0.076)	0.094 ± 0.007 (0.081-0.110)	0.095 ± 0.009 (0.078-0.113)
pt	1.04 ± 0.14 (0.70-1.31)	0.83 ± 0.08 (0.68-1.10)	1.08 ± 0.13 (0.87-1.38)	1.09 ± 0.19 (0.77-1.53)
antIII	0.58 ± 0.06 (0.44-0.66)	0.45 ± 0.06 (0.34-0.56)	0.56 ± 0.07 (0.40-0.70)	0.57 ± 0.09 (0.34-0.72)
rhiantIII	7.5 ± 2.5 (4-16)	3.3 ± 1.4 (0-7)	3.3 ± 1.5 (1-16)	5.0 ± 3.3 (1-16)
bantVI	0.106± 0.013 (0.075-0.122)	0.086 ± 0.009 (0.066-0.108)	0.109 ± 0.013 (0.084-0.013)	0.109 ± 0.009 (0.094-0.132)
siphon	0.21 ± 0.01 (0.19-0.24)	0.17 ± 0.03 (0.10-0.23)	0.26 ± 0.04 (0.19-0.38)	0.27 ± 0.06 (0.17-0.42)
miwsi	0.033± 0.003 (0.025-0.041)	0.024 ± 0.004 (0.018-0.032)	0.026 ± 0.003 (0.021-0.035)	0.024 ± 0.003 (0.016-0.032)
wawsi	0.048± 0.007 (0.035-0.060)	0.029 ± 0.004 (0.023-0.038)	0.040 ± 0.007 (0.025-0.058)	0.034 ± 0.006 (0.023-0.046)
dhasIII	15.4 ± 1.8 (12-19)	6.3 ± 0.7 (5-8)	8.6 ± 1.2 (6-11)	8.2 ± 0.7 (6-10)
abhair	0.079± 0.004 (0.071-0.091)	0.053 ± 0.006 (0.041-0.069)	0.052 ± 0.019 (0.012-0.078)	0.058 ± 0.009 (0.032-0.072)
lurs	0.118± 0.007 (0.104-0.129)	0.086 ± 0.007 (0.069-0.101)	0.109 ± 0.007 (0.092-0.122)	0.112 ± 0.008 (0.087-0.124)
hurs	2.8 ± 0.7 (2-5)	3.6 ± 0.7 (2-5)	4.6 ± 1.0 (3-7)	4.0 ± 0.8 (3-5)

	C. galeopsidis(Rn)	*C. galeopsidis*(Rn/Gt)	*C. maudamanti*
number	28-29	36-39	65-67
body	1.27 ± 0.12 (1.05-1.52)	1.96 ± 0.15 (1.66-2.25)	1.58 ± 0.23 (1.10-2.27)
htibia	1.18 ± 0.13 (0.94-1.36)	1.54 ± 0.15 (1.10-1.87)	1.40 ± 0.17 (0.96-1.66)
tarsII	0.089± 0.006 (0.078-0.099)	0.098 ± 0.008 (0.081-0.113)	0.094 ± 0.008 (0.074-0.115)
pt	1.12 ± 0.10 (0.91-1.33)	1.20 ± 0.14 (0.87-1.45)	1.14 ± 0.12 (0.87-1.31)
antIII	0.50 ± 0.06 (0.37-0.57)	0.61 ± 0.07 (0.45-0.74)	0.56 ± 0.07 (0.38-0.67)
rhiantIII	3.4 ± 1.6 (1-8)	8.6 ± 3.4 (4-16)	3.9 ± 2.3 (0-10)
bantVI	0.091± 0.008 (0.085-0.132)	0.106 ± 0.010 (0.085-0.132)	0.118 ± 0.014 (0.094-0.146)
siphon	0.24 ± 0.03 (0.18-0.31)	0.31 ± 0.04 (0.24-0.38)	0.30 ± 0.04 (0.21-0.38)
miwsi	0.023± 0.004 (0.016-0.037)	0.029 ± 0.002 (0.025-0.035)	0.028 ± 0.003 (0.023-0.035)
wawsi	0.034± 0.005 (0.023-0.042)	0.038 ± 0.007 (0.028-0.058)	0.045 ± 0.007 (0.028-0.062)
dhasIII	6.3 ± 1.2 (4-8)	7.1 ± 1.1 (5-9)	10.1 ± 1.5 (8-14)
abhair	0.060± 0.006 (0.046-0.071)	0.069 ± 0.004 (0.060-0.078)	0.062 ± 0.010 (0.012-0.078)
lurs	0.098± 0.006 (0.087-0.108)	0.109 ± 0.007 (0.097-0.122)	0.117 ± 0.005 (0.101-0.129)
hurs	4.5 ± 0.7 (3-6)	4.5 ± 0.9 (2-6)	4.6 ± 0.9 (3-6)

Table 5.6 alate viviparous females. Morphometric data of *Cryptomyzus* species showing the number of measured specimen, mean ± s.d. and extreme values in parentheses for characters described in Table 5.1.

	C. ulmeri	*C. galeopsidis*(Rr)	*C. galeopsidis*(Rr/Gt)
number	11	17-22	15
body	1.70 ± 0.12 (1.50-1.85)	1.51 ± 0.39 (0.84-2.36)	2.05 ± 0.22 (1.61-2.36)
antIII	0.60 ± 0.05 (0.53-0.69)	0.56 ± 0.08 (0.43-0.70)	0.61 ± 0.02 (0.58-0.67)
antIV	0.41 ± 0.04 (0.35-0.46)	0.41 ± 0.07 (0.30-0.56)	0.47 ± 0.07 (0.35-0.55)
antV	0.43 ± 0.04 (0.36-0.50)	0.38 ± 0.06 (0.30-0.46)	0.41 ± 0.05 (0.36-0.51)
baseVI	0.030± 0.002 (0.027-0.033)	0.026± 0.003 (0.021-0.033)	0.029± 0.001 (0.027-0.032)
pt	1.11 ± 0.11 (0.97-1.26)	1.12 ± 0.12 (0.91-1.29)	1.29 ± 0.17 (1.04-1.56)
srhinIII	22.5 ± 1.9 (18-25)	34.4 ± 6.1 (25-50)	48.7 ± 5.6 (40-58)
srhinIV	9.1 ± 1.6 (6-11)	13.6 ± 4.5 (6-25)	21.4 ± 3.9 (16-28)
siphon	0.20 ± 0.01 (0.18-0.21)	0.25 ± 0.04 (0.17-0.31)	0.25 ± 0.02 (0.22-0.31)
lurs	0.111± 0.005 (0.105-0.119)	0.103± 0.013 (0.070-0.117)	0.124± 0.009 (0.115-0.140)

	C. galeopsidis(Rn)	*C. galeopsidis*(Rn/Gt)	*C. maudamanti*
number	14-17	14-15	18-22
body	1.61 ± 0.18 (1.29-1.87)	1.96 ± 0.28 (1.59-2.60)	1.85 ± 0.31 (1.24-2.25)
antIII	0.58 ± 0.07 (0.49-0.72)	0.63 ± 0.09 (0.47-0.79)	0.58 ± 0.06 (0.40-0.69)
antIV	0.42 ± 0.04 (0.35-0.48)	0.42 ± 0.06 (0.34-0.50)	0.45 ± 0.05 (0.33-0.51)
antV	0.37 ± 0.05 (0.31-0.47)	0.42 ± 0.04 (0.36-0.48)	0.47 ± 0.05 (0.38-0.54)
baseVI	0.027± 0.003 (0.023-0.030)	0.031± 0.004 (0.026-0.037)	0.030± 0.003 (0.026-0.035)
pt	1.22 ± 0.11 (1.05-1.43)	1.40 ± 0.11 (1.22-1.61)	1.18 ± 0.10 (0.98-1.36)
srhinIII	38.9 ± 5.1 (29-46)	57.3 ± 5.1 (47-64)	34.4 ± 4.7 (26-42)
srhinIV	16.6 ± 3.5 (11-21)	25.9 ± 3.3 (19-31)	13.6 ± 4.7 (5-22)
siphon	0.22 ± 0.03 (0.16-0.29)	0.25 ± 0.04 (0.20-0.34)	0.29 ± 0.04 (0.22-0.35)
lurs	0.102± 0.004 (0.096-0.110)	0.113± 0.005 (0.105-0.121)	0.113± 0.022 (0.072-0.131)

Table 5.7 The distribution of *Cryptomyzus* is based on records from the collection of the British Museum (Natural History), London, Great Britain (BM), own data from the Department of Entomology, Agricultural University Wageningen, the Netherlands (AUW) and reports from the following countries (*):
Iceland: Prior & Stroyan (1960), Heie, (1964); Norway: Tambs-Lyche (1970), Stenseth (1971, pers. comm.); Sweden: Ossiannilsson (1959); Finland: Huldén & Heikinheimo (1984), O. Heikinheimo (pers. comm.); Soviet Union: Shaposhnikov (1967), Szelegiewicz (1968), Rakauskas (1980); Poland: Szelegiewicz (1968); Czechoslovakia: Szelegiewicz (1968), Holman & Pintera (1977); Hungary: Szelegiewicz (1969, 1982); Romania: Holman & Pintera (1981); Bulgaria: Grigorov (1980); Italy: Barbagallo & Patti (1985); Switzerland: Lampel (1975), Meier (1975); Spain: Nieto Nafriá et al. (1984, 1987); France: Remaudière (1951, 1952, 1954); the Netherlands: Hille Ris Lambers (1953); Great Britain: Stroyan (1964), L.R. Taylor (pers. comm.); Ireland: Carter et al. (1987); Germany: Börner (1952), Heinze (1961), F.P. Müller and H.A. Eggers-Schumacher (pers. comm.); Denmark: Heie (1962, 1982, pers. comm.); Turkey: Tuatay & Remaudière (1964); Canada: Forbes & Chan (1987); USA: Robinson & Bradley (1968); Mexico: Peña Martinez (1985); Japan: Miyazaki (1971); Korea: Paik (1965).

country	*ribis*	*korschelti*	*ballotae*	*heinzei*	*galeopsidis*	*maudamanti*	*ulmeri*	*alboapicalis*	*leonuri*	*stachydis*
Iceland					* BM AUW					
Norway	*	*			* BM			*		* BM
Sweden	* AUW	*			* BM AUW			* AUW		
Finland	*	*			*					*
USSR (Eur. part)	*	*			*			*	*	
Poland	*	*			*			*	*	*
Czechoslovakia	* BM	*	*	* BM	* AUW	*	AUW	*	* BM	
Hungary	*	AUW		AUW	* AUW		AUW			
Romania	*	*			*					
Bulgaria	*		*							
Turkey	*									
Cyprus			*							
Yugoslavia	BM				BM					
Italy	* BM	*	* BM		*					
Austria	BM						*			
Switzerland		*			*			*		
Spain	*	*	*	AUW	*					
France	*	* AUW	*		BM AUW			* AUW		
Belgium	BM	AUW					AUW			
The Netherlands	* AUW	* BM AUW	AUW		* AUW	AUW	AUW	* AUW		
Great Britain	* BM	* BM	* BM AUW		* BM AUW			* BM AUW		
Ireland	* BM				* BM					
FRG	* BM			AUW	* BM	*	AUW	BM AUW		
GDR	*	*			*		*	*		
Denmark	*	* BM			*			*		
Japan	*									
Korea	*									
Canada	* BM				* BM					
USA	* BM				* BM					
Mexico	* BM									

Key to the European species of *Cryptomyzus*, partly adapted from Hille Ris Lambers (1953).

unwinged viviparous female

1 Hairs on abdominal tergites I - IV, and on median and frontal tubercules on head, shorter than the diameter of the base of antennal segment III, inconspiciously capitate. Alternates between *Ribes rubrum* and *Galeopsis & Stachys*..... *stachydis*
- Hairs on abdominal tergites I - IV, and on median and frontal tubercules on head, usually much longer, on a strong base and clearly capitate.....2
2 Longest hairs on antennal segment III usually shorter than (or equal to) the diameter of the base of antennal segment III, and shorter than the hairs on the Ist segment; last rostral segment at least 1.3 times the second hind tarsus, with 6 - 18 additional hairs (besides the 3 pair near the top); siphunculi > 0.2 times the body..... 3
- Longest hairs on antennal segment III longer than the diameter of the base of antennal segment III, about the same length as those on the Ist segment; last rostral segment at most 1.5 times the second hind tarsus, with 2 - 7 additional hairs (besides the 3 pairs near the top); siphunculi < 0.2 times the body..... 7
3 Anterior abdominal tergites with at most 4 long spino-pleural hairs and 2 long marginal hairs with some very small, inconspicuous knobbed hairs in addition, but usually only with very small and inconspicuous knobbed hairs. On *Ribes alpinum*..... *korschelti*
- Anterior abdominal tergites always with many more long knobbed hairs and absence of very short and inconspicuous hairs.....4
4 Longest hairs on IIIrd antennal segment about as long as the diameter of the segment, only slightly shorter than the longest hair on Ist antennal segment; processus terminalis only 1.2 - 1.6 times the antennal segment III; siphunculi somewhat swollen, mainly on innerside of distal one-third. On *Ballota nigra* and occasionally *Lamium album*, and probably host-alternating with *Ribes alpinum*..... *ballotae*
- Longest hairs on IIIrd antennal segment much shorter than basal diameter of the segment; processus terminalis longer..... 5
5 Siphunculi approximately cylindrical, thinner than the hind tibia; antennal segment III with 0 - 7 rhinaria; last rostral segment < 1.4 times hind tars II; last rostral segment with 6 - 10 additional hairs. Alternates between *Ribes* (*rubrum & nigrum*), and *Stachys palustris*, occasionally on other *Ribes* and *Stachys* species..... *ribis*
- Siphunculi distinctly swollen on distal half, with the largest diameter about 1.4 - 1.6 times the smallest, measured at first half; last rostral segment > 1.4 times the hind tars II; last rostral segment with 8 - 18 additional hairs..... 6
6 Longest dorsal hairs about 3 times the diameter of the hind tibia in the middle; marginal hairs usually in groups of three; antennal segment III with 8 - 18 rhinaria; longest hair in Ist antennal segment thicker and much longer than the longest hair on segment III. Usually on *Stachys sylvatica*.. ..... *korschelti*
- Longest dorsal hairs about twice the diameter of the hind tibia in the middle; marginal hairs usually in groups of two; antennal segment III with 2 - 10 rhinaria; longest hair in Ist antennal segment about the same size as the longest hair on segment III. Alternates between *Ribes alpinum* and *Stachys (Betonica) officinalis*..... *heinzei*
7 Abdominal tergites I - IV each with 12 - 20 hairs.....8
- Abdominal tergites I - IV each with 5 - 14 hairs, if with 12 - 14 hairs, than siphunculi > 0.15 times the body length.....9
8 Antennal segment III with 11 - 22 rhinaria; siphunculi 1.5 - 2.1 times the cauda; processus terminalis < 9 times the base of antennal segment VI. Life cycle not solved, on *Leonurus cardiaca*..... *leonuri*
- Antennal segment III with 5 - 15 rhinaria; siphunculi 1.0 - 1.5 times the cauda; processus terminalis > 9 times the base of antennal segment VI. Monoecious on *Lamium album*..... ..... *alboapicalis*

9 Siphunculi < 0.15 times body; LDF 1 (Table 5.4) < 493.7. Monoecious on *Lamium maculatum* ..... *ulmeri*
- Siphunculi > 0.15 times body; LDF 1 (Table 5.4) > 493.7 10
10 Rostrum reaches up to third pairs of coxae; hairs on I - IVth abdominal tergites 8 - 14; siphunculi with a distinct swollen distal part, relatively long; LDF 2 (table 5.4) < 200.2 (ca 90% of individuals correctly classified). Alternates between *Ribes rubrum* and *Lamium galeobdolon*..... .....*maudamanti* **sp.n.**
- Rostrum shorter; hairs on I - IVth abdominal tergites 4 - 11; siphunculi sometimes not swollen and shorter; LDF 2 (table 5.4) > 200.2 (ca 90% of individuals correctly classified). Alternates between *Ribes* (*rubrum* & *nigrum*) and *Galeopsis*, also monoecious on *Ribes..galeopsidis* Fundatrices of *R. rubrum* and *R. nigrum* forms are distinguishable, but host-alternating and non-alternating forms are not, see section on *C. galeopsidis.*

winged viviparous female

1 Primary rhinarium on Vth antennal segment much larger than secondary rhinaria, > 1.5 times the remaining part of this segment distal to rhinarium.....*stachydis*
- Primary rhinarium on Vth antennal segment about the same size of secondary rhinaria, at most equal to the remaining part of this segment distal to rhinarium.....2
2 Last rostral segment with 6 - 18 additional hairs (besides the 3 pairs near the tip); large, rectangular to trapezium shaped patch on abdomen, usually not or only slightly indented; distinct post-siphuncular patch, if not apparent, than long, cylindrical siphunculi.....3
- Last rostral segment with 3 - 6 additional hairs (besides the 3 pairs near the tip); patch on abdomen strongly indented or only fused stripes present, often one or two stripes more apically; no clear post-siphuncular patch..... 6
3 Processus terminalis up to 2.4 times antennal segment V, no secondary rhinaria on antennal segment V, or few (<5) and then usually on only one of the segments.....*ballotae*
- Processus terminalis > 2.5 times antennal segment V; always both Vth antennal segments with secondry rhinaria (1 - 14).....4
4 Hairs on antennal segment I and III about the same length and much shorter than the diameter of the base of antennal segment III; on the last rostral segment 7 - 9 additional hairs; antennal segment V with 1 - 7 secondary rhinaria.....*heinzei*
- Hairs on antennal segment I about twice as long as those on segment III, longer or slightly shorter than the diameter of the base of antennal segment III; on the last rostral segment 6 - 18 additional hairs; antennal segment V with 3 - 14 secondary rhinaria.....5
5 Siphunculi cylindrical at distal one-third, not attenuated towards the apex; on last rostral segment 6 - 10 additional hairs; antennal segment III with 30 - 46 secondary rhinaria.....*ribis*
- Siphunculi swollen at distal part, attenuated towards the apex; on last rostral segment 11 - 18 additional hairs; antennal segment III with 40 - 60 secondary rhinaria.....*korschelti*
6 Number of hairs on abdominal tergites II - IV is 14 - 20 and siphunculi only slightly longer than the cauda, short and almost not swollen.....*alboapicalis*
- Number of hairs on abdominal tergites II - IV is maximal 14 and if number is between 10 and 14 than siphunculi long and clearly swollen at distal part.....7
7 Number of secondary rhinaria on antennal segment III is 18 - 25 (< 4.4 / 0.1 mm); on the last rostral segment 3 - 4 additional hairs; siphunculi short.....*ulmeri*
- Number of secondary rhinaria on antennal segment III is 26 - 64 (> 5.1 / 0.1 mm); on the last rostral segment 4 - 6 additional hairs; siphunculi usually longer.....8
8 Number of hairs on abdominal tergites II - IV is 9 - 14; secondary rhinaria on antennal segment III is 26 - 42; siphunculi often slightly longer and more pronouncedly swollen at distal part; not all individuals can be classified.....*maudamanti* **sp. n.**
- Number of hairs on abdominal tergites II - IV is 7 - 11; secondary rhinaria on antennal segment III is 25 - 64; siphunculi often shorter and more slender; not all individuals can be classified.....*galeopsidis*

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REFERENCES

Barbagallo, S. & Patti, I. (1985) Remarks on and a check-list of the aphids of the Italian fauna. In: Evolution and Biosystematics of aphids. Proceedings of the International Aphidological Symposium, Jablona, Poland, pp. 393-424. Ossolineum.

Bingham, B. & Sokal, R.R. (1986) Host tree effects on the morphology of *Pemphigus populi-tranversus* (Homoptera: Aphididae). J. Kans. Entomol. Soc. **59**, 287-295.

Blackman, R.L. (1980) Chromosome numbers in the Aphididae and their taxonomic significance. Syst. Entomol. **5**, 7-25.

Blackman, R.L. (1987) Morphological discrimination of a taobacco-feeding form from *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (*Nectarosiphon*) species. Bull. ent. Res. **77**, 713-730.

Blackman, R.L. (1988) Rearing and handling aphids. In: World crop pests. Aphids, their biology, natural enemies and control. volume 2B. (ed. by A.K. Minks, P. Harrewijn), pp. 59-68. Elsevier, Amsterdam.

Blackman, R.L. & Paterson, A.J.C. (1986) Separation of *Myzus (Nectarosiphon) antirrhinii* (Machiati) from *Myzus (N.) persicae* (Sulzer) and related species in Europe (Homoptera: Aphididae). Syst. Entomol. **11**, 267-276.

Börner, C. (1930) Beiträge zu einen neuen System der Blattläuse. Arch. klassif. phylogen. Ent. I, 115-194.

Börner, C. (1952) Europae centralis Aphides. Mitt. thüring. bot. Ges. **3**, 1-488.

Bozhko, M.P. (1961) Novijj rod i novije vidij tlej (Homoptera, Aphididae) s juga Ukrainy, Moldavii i Predkavkoza. Trudy Vses. ent. Obshch. **48**, 5-37.

Brown, P.A. & Blackman, R.L. (1988) Karyotype variation in the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), species complex (Hemiptera: Aphididae) in relation to host-plant and morphology. Bull. ent. Res. **78**, 351-363.

Carter, C.I., Wood-Baker, C.S. & Polaszek, A. (1987) Species, host plants and distribution of aphids occurring in Ireland. Ir. Nat. J. **22**, 266-284.

Dixon, A.F.G. (1974) Changes in the length of appendages and the number of rhinaria in young clones of the sycamore aphid, *Drepanosiphum platanoides*. Entomol. exp. appl. **17**, 1-8.

Docters van Leeuwen, W.M. (1982) Gallenboek. 3d ed. Thieme, Zutphen.

Dunn, G. & Everitt, B.S. (1982) An introduction to mathematical taxonomy. Cambridge University Press, Cambridge.

Eastop, V.F. & Hille Ris Lambers, D. (1976) Survey of the world's aphids. Junk Publishers, The Hague.

El-Gazzar, A. & Watson, L. (1970) A taxonomic study of Labiatae and related genera. New Phytol. **69**, 451-486.

Fargo, W.S., Inayatullah, C., Webster, J.A. & Holbert, D. (1986) Morphometric variation within apterous females of *Schizaphis graminum* biotypes. Res. Popul. Ecol. **28**, 163-172.

Forbes, A.R. & Chan, C.K. (1987) The aphids (Homoptera: Aphididae) of British Columbia. 17. a revised host plant catalogue. J. Entomol. Soc. Brit. Columbia **84**, 72-100.

Grigorov, S. (1980) [Leaf aphids and their control] in Bulgarian. 1-285. Zemizdat, Sophia.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon, L. Weismann), 96-99. SPB Academic Publishing, The Hague.

Guldemond, J.A. (in press) Choice of host plant as factor in reproductive isolation in the aphid genus *Cryptomyzus*. Ecol. Entomol.

Guldemond, J.A. & Eggers-Schumacher, H.A. (1989) Biosystematics of the aphid genus *Cryptomyzus*: an electrophoretic analysis. Z. zool. Syst. Evolut.-forsch. **27**, 14-25.

Gut, J. (1976) Chromosome numbers of parthenogenetic females of fifty-five species of Aphididae (Homoptera) new to cytology. Genetica, **46**, 279-285.

Hampson, M.J. & Madge, D.S. (1986) Morphometric variation between clones of the damson-hop aphid, *Phorodon humuli* (Schrank) (Hemiptera: Aphididae). Agric. Ecosys. Environ. **16**, 255-264.

Hand, S.C. (1986) The use of multivariate morphometric methods in the separation of alate

morphs of the rose-grain aphid *Metopolophium dirhodum*. Ann. appl. Biol. **109**, 19-31.

Heie, O.E. (1962) A list of Danish aphids. Part 3. Ent. Meddr. **32**, 205-224.

Heie, O.E. (1964) Aphids collected in Iceland in August, 1961 (Homopt., Aphididae). Ent. Meddr. **32**, 220-235.

Heie, O.E. (1982) Fund af 25 bladlus arter nye for Danmark (Homoptera: Aphidoidea). Ent. Meddr. **49**, 133-137.

Heikinheimo, O. (1955) A new aphid species *Amphorophora (Ampullosiphon* subg.n.) *stachydis* sp.n. (Hom., Aphididae) from Finland. Suomen hyönt. Aikak. **21**, 5-8.

Heinze, K. (1961) Mitteleuropäische Myzinae. Beitr. Ent. **11**, 54-61.

Hille Ris Lambers, D. (1953) Contribution to a monograph of the Aphididae of Europe V. Temminckia, **9**, 1-176.

Holman, J. & Pintera, A. (1977) Check list Tschechoslowakische Insektenfauna. Acta Faun. Entomol. Mus. Natl. Pragae **15**, supplement 4.

Holman, J. & Pintera, A. (1981) Übersicht der Blattläusen (Homoptera, Aphidoidea) der Rumänischen Sozialistischen Republik. Studie CSAV, Academia, Praha.

Huldén, L. & Heikinheimo, O. (1984) Check list of Finnish insects, Hemipteroidea. Not. Entomol. **64**, 97-124.

Johnson, B. (1959) Effect of parasitation by *Aphidius platensis* Brèthes on the developmental physiology of its host, *Aphis craccivora* Koch. Entomol. exp. appl. **2**, 82-99.

Keep, E. & Briggs, J.B. (1971) A survey of *Ribes* species for aphid resistence. Ann. appl. Biol. **68**, 23-30.

Lampel, G. (1975) Die Blattläusen (Aphidina) des Botanischen Garten Freiburg, Schweiz. Bull. Soc. fribourg. Sc. nat. **64**, 125-184.

Mackay, P.A., Lamb, R.J. & Hughes, M.A. (1989) Sexual and fundatrix-like morphs in asexual Australian populations of the pea aphid (Homoptera: Aphididae). Environ. Entomol. **18**, 111-117.

Meier, W. (1975) Ergänzungen zur Blattlausfauna der Schweiz II. Mitt. schweiz. ent. Ges. **48**, 405-430.

Miyazaki, M. (1971) A revision of the tribe Macrosiphini of Japan (Homoptera: Aphididae, Aphidinae). Insecta Matsum. **34**, 1-247.

Müller, F.P. (1971) Isolationmechanismen zwichen sympatrischen bionomischen Rassen am Beispiel der Erbsenblattlaus *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae). Zool. Jb. Abt. Syst. Oecol. Geogr. Tiere **98**, 131-152.

Müller, F.P. (1982) Das Problem *Aphis fabae*. J. appl. Ent. **94**, 432-446.

Müller, F.P. (1985) Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. Biol. Zbl. **104**, 225-237.

Nieto Nafría, J.M., Diaz Gonzales, T.E. & Mier Durante, M.P. (1984) Catalogo de los pulgones (Homoptera, Aphidoidea) de España y de sus plantas hospendadoras. pp. 174, Universidad de Leon.

Nieto Nafría, J.M., Muñoz Martínez, I. & Mier Durante, M.P. (1987) Pulgones (Hom., Aphidoidea) de la provincia de Soria, II: Aphididae. Bol. Asoc. esp. Ent. **11**, 367-384.

Ossiannilsson, F. (1959) Contribution to the knowledge of Swedish aphids II. list of species with find records and ecological notes. K. LantbrHögsk. annlr. **25**, 375-527.

Paik, W.H. (1965) Aphids of Korea. Seoul National University, Seoul.

Peña Martinez, R. (1985) Ecological notes on aphids of the High Plateau of Mexico, with a check-list of species collected in 1980. In: Evolution and Biosystematics of aphids. Proceedings of the International Aphidological Symposium, Jablona, Poland, pp. 425-430. Ossolineum.

Prior, R.N.B. & Stroyan, H.L.G. (1960) On a new collection of aphids from Iceland. Ent. Meddr. **29**, 266-293.

Rakauskas, R. (1980) [Aphids on fruit-trees and fruit-bearing shrubs of South-East Lithuania.] Liet. TSR Mokslu Akad. Darb. C serija, **2** (90), 33-43.

Remaudière, G. (1951) Contribution à l'étude des Aphidoidea de la faune française. Aphididae: Dactynotinae et Myzinae. Rev. Path. vég. Ent. Agric. Fr. **30** 125-144.

Remaudière, G. (1952) Description de quelques Aphididae nouveaux et addition à la liste des Myzinae et Dactynotinae. Rev. Path. vég. Ent. Agric. Fr. **31**, 232-263.

Remaudière, G. (1954) Deuxième addition à la liste des Dactynotinae et Myzinae (Homoptera, Aphidoidea). Rev. Path. vég. Ent. Agric. Fr. **33**, 232-240.

Robinson, A.G. & Bradley, G.A. (1968) A revised list of aphids of Manitoba. Manit. Entomol. **2**, 60-65.

Robinson, A.G. & Chen Y.A. (1969) Cytotaxonomy of Aphididae. Can. J. Zool. **47**, 511-516.

SAS Institute (1982) User's guide: statistics, 1982 ed. SAS Institute, Inc., Cary, North Carolina.

Shaposhnikov, Ch.G. (1967) In: Key to the insects of the European USSR, vol. 1. (ed. by Bei-Bienko), pp. 787-788. Israel Program for Scientific Translation, Jerusalem.

Sneath, P.H.A. & Sokal, R.R. (1973) Numerical taxonomy. Freeman, San Fransisco.

Stenseth, C. (1971) Morphology and life cycle of *Ampullosiphon stachydis* Heikinheimo (Homoptera, Aphididae). Norsk Entomol. Tidsskr. **18**, 9-13.

Stroyan, H.L.G. (1964) Aphidoidea (excluding Adelgidae and Phylloxeridae). In: A check list of British insects, 2nd edition (eds G.S. Kloet & W.D. Hincks) Roy. Ent. Soc. London, **1**, 67-86.

Szelegiewicz, H. (1868) Katalog Fauny Polski. Mszyce - Aphidoidea. Polska Akademica Nauk. pp. 316.

Szelegiewicz, H. (1968) Faunistisch Übersicht der Aphidoidea (Homoptera) von Ungarn. Fragm. faun. **15**, 57-98.

Szelegiewicz, H. (1982) Beiträge zur Kenntnis der Aphiden fauna (Homoptera, Aphidoidea) von Ungarn. I. Die Aphiden des ungarischen Karstgebietes von Aggtelek. Fragm. faun. **27**, 67-74.

Tambs-Lyche, H. (1970) Studies on Norwegian aphids (Homoptera, Aphidoidea) II. the subfamily Myzinae (Mordvilko) Börner. Norsk entomol. Tidsskr. **17**, 1-16.

Tutay, N. & Remaudière, G. (1964) Première contribution au catalogue des Aphididae de la Turque. Rev. Path. vég. Ent. Agric. Fr. **43**, 243-278.

6 Evidence for sympatric speciation in aphids with particular reference to the genus *Cryptomyzus*

ABSTRACT

Aphids exhibit a strong host plant affinity, cyclical parthenogenesis and a complex life cycle. Several pathways of speciation are described based on these characteristics which are initiated by colonization of a previously unused, new host plant, or the loss of host-alternation. Biological traits of aphids and the speciation pathways open to them, favour the possibility of sympatric speciation in this group of phytophagous insects. The aphid genus *Cryptomyzus* has been selected to illustrate different modes of speciation. Therefore, it was necessary to construct their phylogeny (cladogram), using data on allozymes, life cycle characteristics and morphology. A close association between the phylogeny of this aphid genus and that of its hosts was found. The evolutionary backgrounds for this association, and the modes of speciation in aphids are discussed.

key words: aphid, *Cryptomyzus*, sympatric speciation, host plant shift, phylogeny, host-alternation.

INTRODUCTION

What makes the process of speciation in aphids such a challenging topic? This is due to their complex life cycles, reproductive tactics and strict host plant affinities, which possibly facilitate sympatric speciation in aphids. Possible modes of speciation in aphids have been discussed since the beginning of this century. Mordvilko (1934) was one of the first authors to describe the evolution of the major groups of the Aphidoidea and later Hille Ris Lambers (1950, 1980), Heie (1967), Shaposhnikov (1985) and Müller (1985) have contributed a great deal both to the theory, and the experimental analysis of speciation in aphids. Recently, the genus *Cryptomyzus* Oestlund has been subjected to a biosystematic study (Guldemond 1987, in press, Guldemond & Eggers-Schumacher 1989) and this group is instrumental here to elucidate the different modes of speciation. Firstly, relevant biological characteristics of aphids in general, and of *Cryptomyzus* in particular, will be described.

Aphids differ greatly in their life cycles. Originally, they lived exclusively on woody hosts (Heie 1967). In about 10% of the present species host-alternation between two taxonomically unrelated host plants has evolved (Eastop 1973). The primary or winter host is usually a woody plant on which sexual reproduction occurs, and the secondary or summer host is usually a herbaceous plant in the Aphididae, although in other families this too may be a woody host (Dixon 1985). A so-called secondary monoecious species evolved on the secondary host due to the loss of host-alternation (Hille Ris Lambers 1950; Blackman & Eastop 1984). Apparently, host-alternation is a factor which has significant implications for speciation of aphids.

Secondly, aphids are cyclically parthenogenetic combining predominantly parthenogenetic reproduction with sexual propagation in a one-year cycle. Two-year cycles have also been discovered (Heie 1980). There are two ways of producing sexuals in host-alternating aphids. In the Aphididae males and autumn migrants (gynoparae) return from the secondary to the primary host where the autumn migrants produce the sexual oviparous females. This generally results in outbreeding. In the more primitive Pemphigidae, Anoeciidae and Hormaphididae (Heie 1987) the autumn migrants (sexuparae) produce both males and oviparae on the primary host (Hille Ris Lambers 1966). This results generally in inbreeding.

The consequence of parthenogenesis is a rapid population growth, which is in-

creased even further by vivipary, telescoping of generations and winglessness. This leads to an eightfold greater reproductive rate than can be achieved by a sexual reproducing, winged female (Dixon, in press). Sexual reproduction in aphids usually takes place in autumn and assures genetic heterogeneity by recombination and segregation (Kirkpatrick & Jenkins 1989).

A third feature of aphids is their strict host specificity. Eastop (1973) demonstrated that 99% of aphid species is restricted to one or a few closely related host plants. This indicates that the host on which sexual reproduction occurs, may be an important factor in the reproductive isolation of closely related forms and their subsequent speciation (Guldemond, in press).

Cryptomyzus is a good genus in which to study the differentiation of closely related forms. In Europe 13 different species and forms are found, which are either host-alternating between the currant, *Ribes*, and several herbaceous species of Labiatae, or non-alternating (Guldemond 1987). In the *C. galeopsidis* (Kaltenbach)/*C. alboapicalis* (Theobald) complex morphologically very similar forms and species occur, which (1) differ in life cycle: either host-alternating, or non-alternating on secondary or primary host; (2) differ in primary host, but share the same secondary host; or (3) differ in secondary host, but share the same primary host (Figure 6.1). These forms have been demonstrated to be either separate species, host races or different conspecific life cycle forms (Guldemond & Eggers-Schumacher 1989; Guldemond, in press & chapter 5).

This study addresses the questions: Which biological characteristics of aphids would favour sympatric speciation in this group? Which patterns of speciation, represented by the phylogeny, might be involved in *Cryptomyzus*? Subsequently, the process of speciation in *Cryptomyzus* will be derived and extrapolated to other aphid groups as well.

Phylogeny of *Cryptomyzus*

Hille Ris Lambers (1953) provided a biological and taxonomic survey of *Cryptomyzus*, which was extended by Guldemond (1987 & chapter 5). A phylogeny based on allozyme data was given by Guldemond & Eggers-Schumacher (1989). Incorporation of data on morphology and life cycle, and using *C. (Ampullosiphon) stachydis* (Heikinheimo) as an

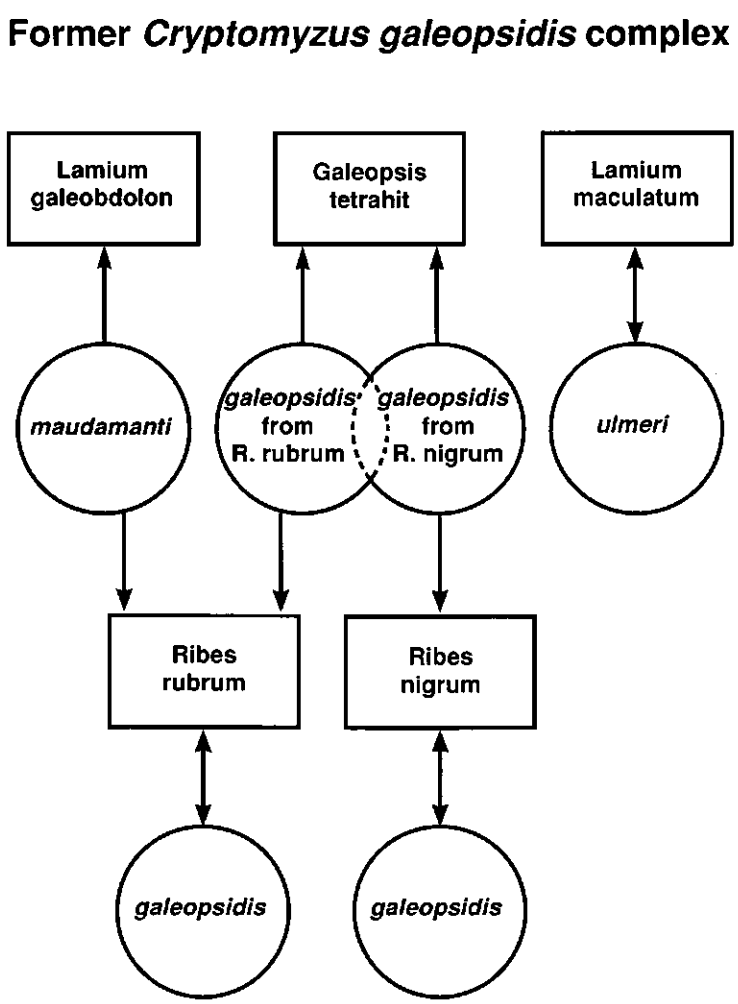

Figure 6.1 Life cycles and host plants of the species of the former *Cryptomyzus galeopsidis* complex.

out-group, resulted in a cladogram for *Cryptomyzus* (Figure 6.2). The apomorphic (derived) characters, which define the branches (clades), are defined by numbers on the tree and listed in Table 6.1.

At the base of the tree is *C. (A.) stachydis* with only very short slightly capitate hairs, similar to those of the closly related *Nasonovia* species (Guldemond & Eggers-Schumacher 1989). All other *Cryptomyzus* species share the same apomorphic character in that the wingless morphs have long, capitate hairs each on a strong base, situated on head, thorax and abdominal tergites. *C. galeopsidis*, *C. alboapicalis* and *C. ulmeri* (Börner) share the apomorphic character of long hairs on the antennae, and Börner (1930) erected a new subgenus, *Myzella*, for these species. *C. leonuri* Bozhko and *C. maudamanti* Guldemond also belong to this group (chapter 5). Evidence for a monophyletic origin of this group is also provided by allozyme data (Guldemond & Eggers-Schumacher 1989). The species *C. ulmeri*, *C. maudamanti* and *C. galeopsidis* are closely related, because they have fewer hairs on the abdominal tergites, but the exact position of *C. leonuri* is not yet clear. A close relationship between *C. korschelti* Börner and *C. ribis* (L.), and *C. ballotae* HRL and *C. heinzei* HRL, respectively, was supported by allozyme data, but only a few apomorphic characters were found to support the lower branch which includes all these species. No attempt was made to determine a phylogeny based only on morphology, because most of these characters display an almost continuous pattern of variation among the different species.

When the (characteristic) secondary host plants of the *Cryptomyzus* species are superimposed on the derived phylogeny, the grouping of plants reflects their taxonomy (Figure 6.3). All secondary hosts belong to the subtribe *Lamiinae* (Briquet 1897), which forms only a small part of the Labiatae (Lamiaceae). Further, species of *Lamium*, *Galeopsis* and *Leonurus*, which are used by related *Cryptomyzus* species, are combined in one group, as well as *Stachys* and *Ballota* (El-Gazzar & Watson 1970). Obviously, taxonomically related host plants are colonized by related species of *Cryptomyzus*.

Why are aphids good candidates for sympatric speciation?

Sympatric speciation in phytophagous insects is often considered to commence with the appearance of host races (Bush 1975; Menken 1981; Diehl & Bush 1984). Jaenike

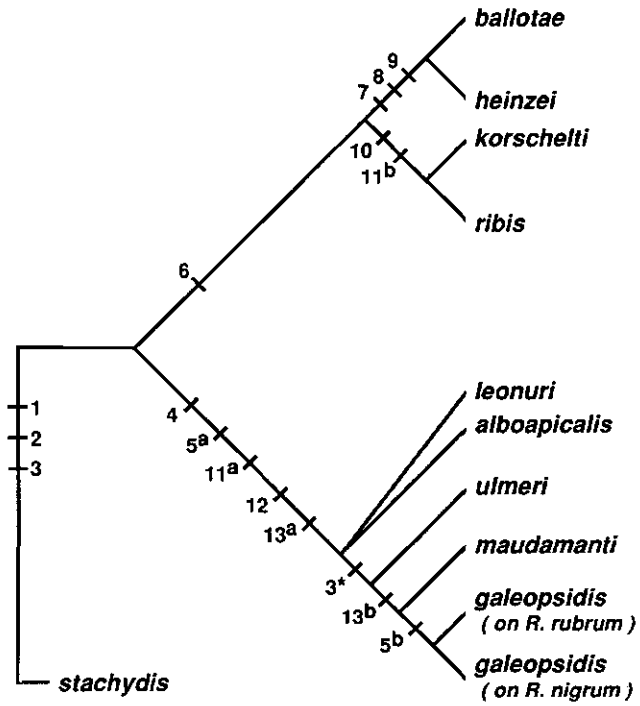

Figure 6.2 Cladogram of the European *Cryptomyzus* species, based on allozymes and life cycles. Apomorphic characters defining the clades (not the species) are indicated on the tree and specified in Table 6.1.

Table 6.1 The plesiomorphic and apomorphic characters used for the cladogram of *Cryptomyzus* (Figure 6.2). * homoplaseous character. Electromorphs as in Guldemond & Eggers-Schumacher (1989).

	plesiomorphic characters	apomorphic characters
1	short abdominal hairs	long abdominal hairs
2	slightly capitate hairs	strongly capitate hairs
3	dorsal hairs on abdominal tergite III ≤ 11	idem > 11
4	antennal hairs < diameter of the base of antennal segment III	idem >
5	host-alternation	5^a allele for monoecy; 5^b monoecy on primary host
6	primary host plant: *Ribes rubrum/nigrum*	primary host plant: *Ribes alpinum*
7	6-Pgdh^C	6-Pgdh^B
8	Sdh^C	Sdh^B
9	Gpdh^C	Gpdh^A
10	Idh-1^A	Idh-1^B
11	Hk-1^C	11a Hk-1^A; 11b Hk-1^D
12	G-6-Pdh^A	G-6-Pdh^C
13	Pgi^E	13a Pgi^E; 13b Pgi^G

(1981) specified the conditions for ascertaining the existence of host races. The first step in the formation of a host race is the colonization of a new, previously unused, host plant which is assumed to be initiated by a change in the insects host preference (Futuyma 1983; Jermy 1987). Then its reproductive performance should be adapted to the new type of food.

Aphids exhibit many of the features thought to be important for the formation of host races and are therefore suitable candidates for sympatric speciation. These features of aphids are described below and compared with the situation more commonly encountered in phytophagous insects.

host specificity

* Most aphids are highly host specific (Eastop 1973), a characteristic that is shared by other groups as well. However, because the sexual female is usually wingless and

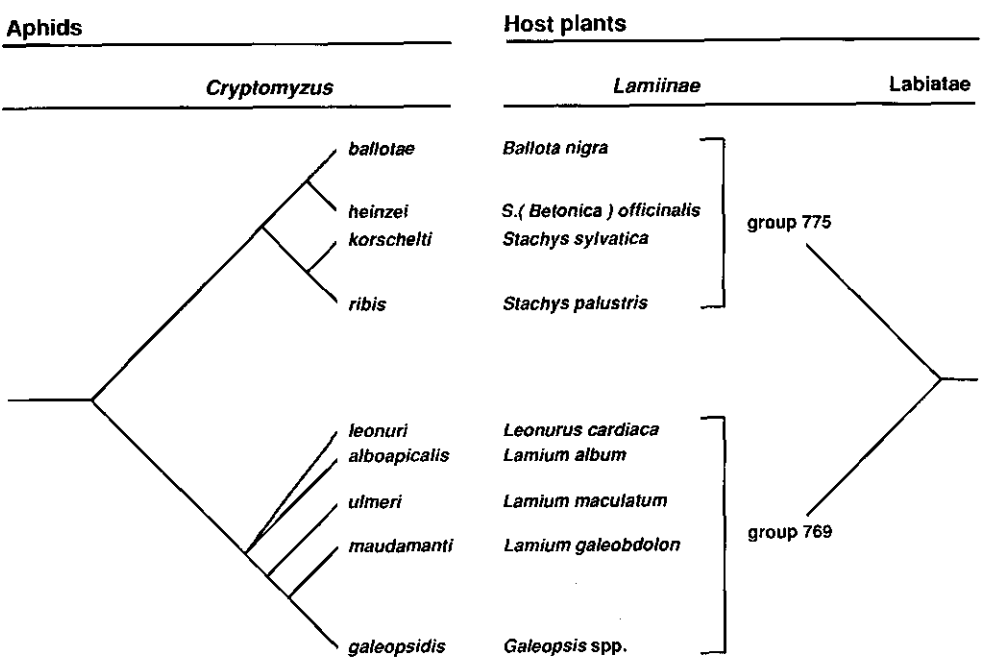

Figure 6.3 Association between the phylogeny of *Cryptomyzus* and that of its labiateous host plants. Group 775 and 769 as in El-Gazzar & Watson (1971).

occasionally also the male, mating always occurs on the host. In the case of other host-specific insects males and females are usually winged and mating may occur on or near the host plant, e.g. in fruit flies, *Rhagoletis* (Prokopy et al. 1971) and ermine moths, *Yponomeuta* (Menken 1981).

* Host plant preference is well developed and may lead to reproductive isolation between closely related forms (Müller 1985; Guldemond, in press).

* Host plant preference and reproductive performance are genetically determined (Müller 1985; chapter 4) and, because of the strict association with a particular host plant, gradual physiological adaptation (induction) to another host plant is unlikely (chapter 2, but see Shaposnikov 1966).

cyclical parthenogenesis

* If a mutation occurs which changes host preference, in order to colonize a new host plant only a single female is required, instead of a mated female as in a sexually reproducing species. This individual aphid can give rise to a new clone, bearing this mutation, by parthenogenetic propagation. The mutated allele(s) should have some degree of dominance to be expressed instantly.

* The offspring are genetically identical to the mother, therefore they all reveal the mutated (host preference) trait. The offspring of a sexual reproducing species share only half of their mothers' genome and their host preference for the new host may be less distinct.

* The number of mutant aphids on the new host may increase rapidly by parthenogenetic reproduction, vivipary and telescoping of generations (Dixon, in press). In a sexually reproducing species fewer generations are possible. A larger population will have a greater chance of survival.

* Due to the fact that in most cases close inbreeding takes place, the probability of fixation of the new character by the formation of homozygotes is great, because half of the gametes of sexual daughters and sons share the same genetic material as their mother. Heterozygous loci, which regulate viability and preference for the new host, may become homozygous by means of recombination and segregation. Consequently the performance on the new host may improve. A small part of the sexually produced descendants would have the homozygous condition of preference for the original host plant. These individuals in turn may return to this host, which would result in a one-way de-

veloped gene flow from the new to the old host plant. Another conceivability could be that these individuals do not survive on the new host leading to the elimination of this genotype.

Pathways of speciation in aphids

To facilitate speciation after a shift to a new host plant, the population with the newly acquired characters (host preference) should become reproductively isolated from its original population to escape introgression. The question is how this reproductive isolation could be established? Several options (see also Ward 1987) are described below.

non-alternating species

A non-alternating (monoecious) species living on a herbaceous plant colonizes a new host. Reproductive isolation from the original population might result because the sexuals are both formed on the new host and no sexuals of the original population will settle on this host (Figure 6.4A). A prerequisite would be that the new form should lose its preference for the original host plant. This is not an unlikely event because in order to colonize the new host, a change in preference should have already occurred. Reproductive performance on the original host may or may not have changed, but presumably the new form would gradually adapt to the new host and concomitantly reproductive performance on the previous host would be reduced.

Another possibility for speciation is the origin of a monoecious form from a host-alternating species (Figure 6.4B). Assume that a mutant colonizes a new secondary host and concurrently looses host-alternation when it is "captured" on this host (Hille Ris Lambers 1950; Ward 1987). Some evidence supports the argument that in *C. galeopsidis* host-alternation is genetically determined by only one gene (complex) (chapter 4). If this represents a more general phenomenon, the colonization of a new host by a female which is heterozygous for host-alternation, followed by the production of sexuals, would partially produce homozygous non-alternating offspring by segregation. Once more, a new form is "captured" on a new secondary host and is instantaniously reproductively isolated from its ancestor. In genera lacking the combination of both host-alternating and non-alternating forms, only one mutation is required therefore establishing an allele for

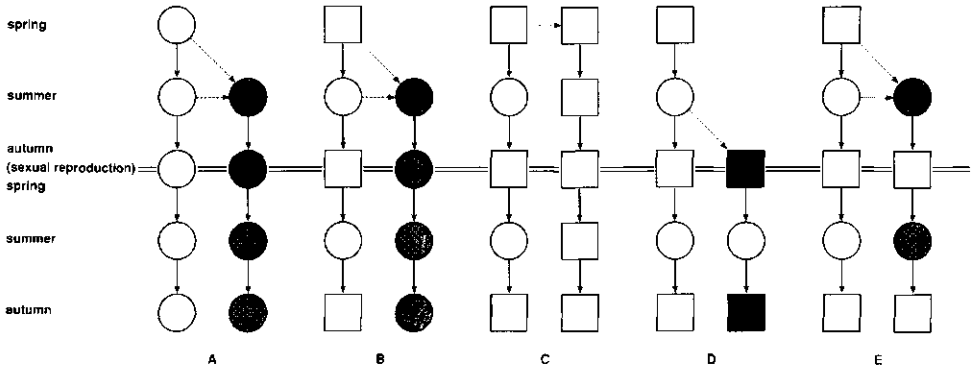

Figure 6.4 Possible pathways of speciation in aphids by means of colonization of a new host plant. Squares represent primary host plants, circles secondary host plants; a solid arrow represent the normal life cycle, an interrupted arrow colonization of a new host plant.

non-alternation.

A monoecious species may also arise on the primary host by the loss of host-alternation (Figure 6.4C). A good example of this is to be found in the Hormaphididae where differences in distribution (altitude) and appearance of sexuals were found to exist between monoecious and heteroecious forms (von Dohlen & Gill 1989).

Finally, new species may originate by losing their sexual mode of reproduction and becoming completely anholocyclic (Ward 1987). In this case reproductive isolation is no longer a problem. Several such species have been described. In the host-alternating Fordini no secondary monoecious species have been evolved and only anholocyclic forms have permanently colonized secondary host plants (Heie 1980).

host-alternating species

Assume that in a host-alternating species, living on its secondary host, a mutation occurs, which causes a female to prefer a new primary host. In autumn, females (gynoparae) as well as males are produced with an altered primary host preference. Both these morphs would colonize the new primary host, where oviparae are produced and inseminated by the males (Figure 6.4D). Sexuals of the original population do not colonize the

new host and gene flow at least is strongly reduced. Furthermore, the chance that both mutated females and males would arrive on the same individual of the primary host and mate, is minimal. Therefore this mode of speciation is probably rare.

Another situation is encountered when a host-alternating species colonizes a new secondary host. On returning to the primary host in the autumn, several options are open: (1) normal interbreeding between both forms occurs which extends the range of secondary host plants with no consequences for speciation; (2) interbreeding is reduced due to the development of assortative mating (Maynard Smith 1966) or because of the low viability of the hybrids of the two host forms (hybrid inferiority). The original and new populations separate, due to the reduced gene flow between them (Figure 6.4E). What will happen depends on the fitness of the new population on both its original and new host. If a good reproductive performance is achieved on both host plants, the host range is extended. If on the other hand a good performance on the new host is associated with a poor performance on the original host, selection will favour a more intense relationship with the new host (Via 1986; Ward, in press). A reduction in the fecundity of the hybrids would cause a more marked selection for a premating isolation barrier, corresponding to assortative mating by mate preference.

DISCUSSION

Relation between the phylogeny of aphids and their host plants

It is already known that many groups of taxonomically related aphids have colonized taxonomically related host plants, although there are several exceptions (Hille Ris Lambers 1950 & 1980; Eastop 1973 & 1986; Heie 1980). In other phytophagous insect groups similar cases are known (Zwölfer & Herbst 1988). Little information is available on the phylogeny of both aphids and their host plants. Among the aphid species of the genus *Cryptomyzus* just such a correlation with the phylogeny of their hosts was discovered (Figure 6.3). The plant genera exploited by European *Cryptomyzus* all belong to the subtribe *Lamiinae*, and vice versa all European *Lamiinae* are hosts to *Cryptomyzus*. This plant taxon includes another genus, *Phlomis*, on which two Asian species of *Cryptomyzus* live (Remaudière & Davitchi 1961; Narzikulov & Daniarova 1979). A third spe-

cies from East Asia and Japan, *C. taoi* HRL, lives on a related genus *Marrubium* (El-Gazzar & Watson 1970).

This raises the question: is this association the result of coevolution, which would imply a reciprocal cladogenesis of aphid and host (Thompson 1989). In general, it is unlikely that of the multitude of interactions influencing the fitness of a plant, the action of one of the herbivores could exert such an intense selection pressure that it would cause speciation of the plant (Jermy 1984). It therefore seems more plausible that aphids follow the evolution of plants, which Jermy calls sequential evolution.

This does not answer the question: why are related species of plant often colonized by closely related aphids? A new host should be suitable for an aphid in terms of: (1) chemical characteristics, which determine the nutritional suitability, and secondary plant compounds, which may serve as olfactory or gustatory attractants/deterrents (e.g. Visser & Taanman 1987); (2) morphology and anatomy, for instance hairs or the position of the phloem may prevent or enable aphids to feed (Heie 1980; Moran 1986); (3) the phenology of the host and its ecology, should at least partly match those of the aphid; (4) abundance of the host, because rare plants are seldom colonized (Dixon et al. 1987). In addition, a new host on which mating of the sexuals occurs, should have suitable sites for egg laying, survival and hatching.

It is evident that there are many, unrelated plant species, living in similar habitats as that of the original host plant and having a similar phenology and abundance. It seems reasonable to assume that in particular chemical, anatomical and morphological characteristics of the new host are more likely to be similar to those of the original host, if they are related to each other. The chemical similarity may especially be important because a mutation of the host preference genes ought to change the aphids receptor mechanism. This may lead to the perception of closely related chemical compounds, which are more likely to be found in a related host plant. Although this may explain the often recognized association between related aphids and hosts, it does not however exclude host shifts to completely unrelated plant families (Hille Ris Lambers 1950).

When a small number of genes code for host preference, the frequency of a change in preference might be greater than when it is controlled the presence of many genes. In swallowtail butterflies there are indications that only a limited number of genes determine oviposition preference (Thompson 1988) and Ward (in press) postulates that in aphids at least three genes are involved. In *Rhagoletis* there have been recent

changes in host preference, indicating that this phenomenon is not rare (Bush 1975). In aphids host plant relationships can be very old (Moran 1989), but the large number of closely related, morphologically similar aphids, which differ solely in their host plants (Müller 1986), also suggests recent changes in host preference (Blackman 1981).

Sympatric speciation in *Cryptomyzus*

The work of Bush (1969) on sympatric speciation of the fruit fly *Rhagoletis*, renewed a lively debate on sympatric speciation. Futuyma & Mayer (1980) and Jaenicke (1981) criticized the available evidence for host race formation, and consequently sympatric speciation, nevertheless convincing data are accumulating (e.g. Feder et al. 1988; Smith 1988). In 1971 Müller first mentioned the possibility of sympatric speciation in the context of aphids for the complex of *Acyrthosiphon pisum* (Harris). Several of its forms are reproductively isolated on different hosts, despite the potential to hybridize under experimental conditions.

Whether the case being sympatric or allopatric speciation is not the real issue, as this is partly a semantic discussion. A major question to face, when considering speciation in highly mobile phytophagous insects, is whether it is possible to colonize and adapt to a new host, when the mobility of the insect makes gene flow with the original population likely?

The origin of the different taxa in the species complex of *Cryptomyzus galeopsidis* (Figure 6.1) could be accounted for in terms of sympatric speciation. All forms, which are now considered species, *C. ulmeri* and *C. maudamanti* (chapter 5), morphologically close to *C. galeopsidis*, occur sympatrically, and have recently speciated. The distinct host preferences of the taxa enable the separation between *C. ulmeri*, restricted to *Lamium maculatum*, from all other taxa. The host races of *C. galeopsidis* migrating to *Ribes rubrum* and *R. nigrum*, respectively, also differ in host preference and reproductive performance, leading to diminished gene flow as concluded from differences in allozyme frequences (Guldemond & Eggers-Schumacher 1989; Guldemond, in press).

It is impossible to prove that a new population did not originate allopatrically. Nevertheless, the distribution of the secondary host plants *L. maculatum* and *L. galeobdolon* overlap almost completely, and *Galeopsis* species cover the same area but show

an extention further North and East (Meusel et al. 1978). As *Galeopsis* seems to be the original host for this clade, the overlapping distributions of the host plants does not indicate varying centres of origin. Additionally, the distribution of the primary host plants *Ribes rubrum* and *R. nigrum* are more or less congruent (Komarov 1939). A sympatric origin for *C. maudamanti* is less likely, because it shares its primary host, *R. rubrum*, with *C. galeopsidis*. However, if *R. nigrum* has been the original host for this clade, then *C. maudamanti* may have been the first colonizer of *R. rubrum*. Later, after divergence of this form, a new colonization took place with the emergence of the *R. rubrum* form of *C. galeopsidis*.

Pathways of speciation

non-alternating species

The so-called secondary monoecious species (Hille Ris Lambers 1950), which are derived from host-alternating ones, are a successful group. Within the Aphididae, whose ancestors are supposed to have been host-alternating (Hille Ris Lambers 1950), several genera are confined to herbaceous plants, e.g., *Macrosiphoniella* del Guerico on Compositae, *Acyrthosiphon* Mordvilko on several plant families and *Uroleucon* Mordvilko on Compositae and Campanulaceae (Hille Ris Lambers 1938, 1939 & 1947). It should be noted that some of these presumed monoecious species actually host-alternate between herbaceous plants (Müller & Hubert-Dahl 1973; Moran 1983), which indicates that the meeting of the sexuals is a critical phase in the life cycle of aphids (Ward 1987).

It is difficult to determine whether monoecious species all originated from host-alternating ancestors (Figure 6.4B) or from monoecious species (Figure 6.4A). This can only be solved when the phylogeny of a genus with both host-alternating and non-alternating species is unravelled. *Cryptomyzus* is such a genus, and its phylogeny does not support the origin of the non-alternating species on *Lamium* from other monoecious species (Figure 6.2). A more plausable explanation for the origin of these monoecious forms is that they are derived from host-alternating species (Figure 6.4B). The ancestor of the branch of the phylogenetic tree, which includes these taxa (Figure 6.2), is thought to have been host-alternating.

In the Aphididae a problem is encountered with this mode of speciation because

the sexual females (oviparae) are produced by the autumn migrants (gynoparae) which return to the primary host. This implies that in general no oviparae would remain on a newly colonized secondary host. However, it was occasionally observed that oviparae are produced on the secondary host as in the case of *C. galeopsidis* on its host *Galeopsis tetrahit* (Guldemond, unpublished results). Also *Myzus persicae* (Sulzer) produces oviparae on the secondary host plant oilseed rape, *Brassica napa* L. (F.L. Dieleman, pers. comm.). Because males are always produced on the secondary host, mating could then occur there. This is not a problem in Pemphigidae because males and sexual females are both produced by sexuparous females at the same place on the primary host.

Reproductive isolation of a monoecious form restricted to the primary host, which was derived from a host-alternating species with the same primary host (Figure 6.4C), is not likely to be achieved sympatrically. The origin of the monoecious form of *Hormaphis hamamelidis* (Fitch) is probably due to the lack of the secondary host at higher altitudes, where this form is found, but it does overlap with the host-alternating form. An earlier appearence of the sexuals also reduces gene flow (von Dohlen & Gill 1989).

In *C. galeopsidis* non-alternating forms occur on the two primary hosts of the heteroecious forms (Figure 6.1; Hille Ris Lambers 1953; Guldemond 1987). The sexuals of the monoecious forms are produced earlier in the season (Hille Ris Lambers 1953), but do overlap with those of the host-alternating forms and there is no reason to separate them taxonomically (chapter 5). Nevertheless, some inbreeding of the monoecious form is likely to occur, and due to the absence or scarcity of secondary hosts a distinct form may evolve. Allochronic isolation also seems to contribute to the origin and persistence of *Acyrthosiphon pisum destructor* Johnson, whose sexuals appear much later in the year compared with other forms (Müller 1980).

Monoecy on the primary (woody) host is presumed to be the primitive (plesiomorphic) condition in aphids (Heie 1967). When this condition occurs in a group of host-alternating species it is probably a derived character. This was concluded for monoecious species of Pemphigidae (Aoki & Kurosu 1988) and the Hormaphididae (von Dohlen & Gill 1989; von Dohlen, in press). This is also likely to be the case in the non-alternating forms of *C. galeopsidis*, because host-alternation is a plesiomorphic character in *Cryptomyzus* (Figure 6.2).

host-alternating species

Speciation by way of colonization of a new primary host (Figure 6.4D) has occurred in many aphid groups. In *Cryptomyzus* the colonization of *Ribes alpinum* led to the origin of three species (Figure 6.2). The host races of *C. galeopsidis* on *Ribes rubrum* and *R. nigrum* probably arose from the differentiation in host plant preference and reproductive performance, although hybrids between these two forms exist in nature (Guldemond, in press). Also in *Myzus cerasi* F. two closely related "subspecies" inhabit different primary host plants (Dahl 1968), but they differ in allozyme pattern (Gruppe 1988) and probably represent separate species.

A successful mode of speciation in aphids has been the colonization of a new secondary host while retaining the original primary host (Figure 6.4E). In many aphid genera the various species inhabit a few, often similar, primary hosts but all have different secondary hosts. Examples are, seven species of *Cryptomyzus* which all have different secondary hosts, but only use three species of *Ribes* as primary hosts, or the 20 *Dysaphis* species in Europe and the Caucasus with 20 different secondary hosts, but only using 5 *Malus* and 2 *Crataegus* species as primary host (Stroyan 1958 & 1985). Moreover sibling species often share a primary host, e.g. *Cryptomyzus galeopsidis* and *C. maudamanti* on *Ribes rubrum* (Figure 6.1). Reproductive isolation between such forms may arise by hybrid inferiority which is manifested by a reduced reproductive performance and host finding ability (chapter 4). A similar case is described by Müller (1985) who demonstrated differences between two sibling species in the number of pheromone producing glands, which possibly indicates that premating barriers have evolved (Müller & Hubert-Dahl 1979).

In the Pemphigidae one may expect a more frequent colonization of new primary host plants compared with the Aphididae, because of inbreeding due to the simultaneous production of sexuals by one female. However, this is not the case: 9 Dutch *Pemphigus* species all use different secondary hosts, but 7 of them share *Populus nigra* as their primary host plant (Heie 1980; J. Prinsen, pers. comm.).

This indicates that the successful colonization of a new primary host is hampered by other factors than the chance of both sexes meeting on the new host (see Ward 1987). It is remarkable that in the Pemphigidae no secondary monoecious species have been formed on herbaceous plants (Hille Ris Lambers 1980), other than permanent anholocyclic forms (Heie 1980). The suggested specificity of the fundatrix, which is possibly

not able to survive on a new host, may be crucial here (Shaposhnikov 1985; Moran 1988). Whether this holds only for Pemphigidae and related primitive aphids, or for the more modern Aphididae as well, is unknown, but the enormous radiation of the Aphididae on herbaceous plants suggests different patterns of evolution in these groups of aphids. Apparently, there are several ways in which speciation could occur in aphids, and the data on species complexes and biology of aphids suggests that sympatric speciation is one of the possibilities.

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REFERENCES

Aoki, S. & Kurosu, U. (1988) Secondary monoecy of a North American gall aphid, *Pemphigus monophagus* (Homoptera, Aphidoidae). Kontyu **56**, 394-401.

Blackman, R.L. (1981) Species, sex and parthenogenesis in aphids. In: The evolving biosphere. (ed. P.L. Forey), pp. 77-85. Cambridge University Press, Cambridge.

Blackman, R.L. & Eastop, V.F. (1984) Aphids on the world's crops. John Wiley & Sons, Chichester.

Briquet, J. (1897) In: Engler, A. & Prantl, K. Die natürlichen Pflantzenfamilien IV. Teil 3, Abteilung a. Engelman Verlag, Leipzig.

Bush, G.L. (1969) Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). Evolution **23**, 237-251.

Bush, G.L. (1975) Sympatric speciation in phytophagous parasitic insects. In: Evolutionary strategies of parasitic insects and mites. (ed. by P.W. Price), pp. 187-206. Plenum, New York.

Dahl, M.L. (1968) Biologische und morphologische Untersuchungen über den Formenkreis der schwarzen Kirchenlaus *Myzus cerasi* F. (Homoptera: Aphididae). Dt. ent. Z., N.F. **15**, 281-312.

Diehl, S.R. & Bush, G.L. (1984) An evolutionary and applied perspective of insect biotypes.

Ann. Rev. Entomol. **29**, 471-504.

Dixon, A.F.G. (1985) Aphid ecology. Blackie, Glasgow, pp. 168.

Dixon, A.F.G. (in press) Evolutionary aspects of parthenogenetic reproduction in aphids. In: Proceedings of the III. International Symposium on Aphids, 1989, Kecskemet, Hungary.

Dixon, A.F.G., Kindlemann, P., Leps, J. & Holman, J. (1987) Why are there so few aphid species, especially in the tropics? Am. Nat. **129**, 580-592.

Eastop, V.F. (1973) Deductions from the present day host plants of aphids and related insects. In: Insect/plant relationships. (ed. by H.F. van Emden), pp. 157-178. Symp. Roy. Ent. Soc. London **6**, London.

Eastop, V.F. (1986) Aphid - plant associations. In: Coevolution and systematics. (ed. by A.R. Stone & D.L. Hawksworth) pp. 35-54. The systematic Association, special volume **32**.

El-Gazzar, A. & Watson, L. (1970) A taxonomic study of Labiatae and related genera. New Phytol. **69**, 451-486.

Feder, J.L., Chilcote, C.A. & Bush, G.L. (1988) Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella*. Nature **336**, 61-64.

Futuyma, D.J. (1983) Selective factors in the evolution of host choice by phytophagous insects. In: Herbivorous insects: host seeking behaviour and mechanisms. (ed. by S. Ahmad), pp. 227-244. Academic Press, New York.

Futuyma, D.J. & Mayer, G.C. (1980) Non-allopatric speciation in animals. Syst. Zool. **29**, 254-271.

Gruppe, A. (1988) Elektrophoretische Untersüchungen zur Unterscheidung der Subspecies von *Myzus cerasi* F. (Hom., Aphididae). J. appl. Ent. **105**, 460-465.

Guldemond, J.A. (1987) Biosystematics of the *Cryptomyzus* complex. Preliminary results on host plant acceptance, electrophoretic data and sexual isolation. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 96-99. SPB Academic Publishing, The Hague.

Guldemond, J.A. (in press) Choice of host plant as factor in reproductive isolation of the aphid genus *Cryptomyzus* (Homoptera, Aphididae). Ecol. Entomol.

Guldemond, J.A. & Eggers-Schumacher H.A. (1989) Biosystematics of the aphid genus *Cryptomyzus* (Homoptera, Aphididae): an electrophoretic analysis. Z. zool. Syst. Evolut.-forsch. **27**, 14-25.

Heie, O.E. (1967) Studies on fossil aphids (Homoptera: Aphidoidea). Spolia Zool. Mus. Haun. **26**, 1-274.

Heie, O.E. (1980) The Aphididae of Fennoscandia and Denmark. I. Fauna Ent. Scand., vol. **9**, 1-236.

Heie, O.E. (1987) Paleontology and phylogeny. In: World crop pests. Vol. 2A. Aphids, their

biology, natural enemies and control. (ed. by A.K. Minks & P. Harrewijn), pp. 367-391. Elsevier, Amsterdam.

Hille Ris Lambers, D. (1938) Contribution to a monograph of the Aphididae of Europe I. Temminckia **3**, 1-44.

Hille Ris Lambers, D. (1939) Contribution to a monograph of the Aphididae of Europe II. Temminckia **4**, 1-134.

Hille Ris Lambers, D. (1947) Contribution to a monograph of the Aphididae of Europe III. Temminckia **7**, 179-319.

Hille Ris Lambers, D. (1950) Host plants and aphid classification. Proc. 8th Int. Cong. Entomol., Stockholm, pp. 141-144.

Hille Ris Lambers, D., 1953. Contribution to a monograph of the Aphididae of Europe V. Temminckia **9**: 1-176.

Hille Ris Lambers, D. (1966) Polymorphism in Aphididae. Ann. Rev. Entomol. **11**, 47-78.

Hille Ris Lambers, D. (1980) Aphids as botanists? Symb. bot. Upsal. **12**, 114-119.

Jeanicke, J. (1981) Criteria for ascertaining the existence of host races. Am. Nat. **117**, 830-834.

Jermy, T. (1984) Evolution of insect/plant relationships. Am. Nat. **124**, 609-630.

Jermy, T. (1987) The role of experience in the host selection of phytophagous insects. In: Perspectives in chemoreception and behavior. (ed. by R.F. Chapman, E.A. Barnays & J.G. Stoffolano, Jr.), pp.143-157, Springer-Verlag, New York, Inc.

Kirkpatrick, M. & Jenkins, C.D. (1989) Genetic segregation and the maintenance of sexual reproduction. Nature **339**, 300-301.

Komarov, V.L. (1939) Flora of the U.S.S.R., Vol. IX. Botanical Institute of the Acadamy of Sciences of the USSR. Scientific Translation Program, Jerusalem, 1971.

Maynard Smith, J. (1966) Sympatric speciation. Am. Nat. **100**, 637-650.

Meusel, H., Jäger, E. & Weinert, E. (1978) Vergleichende Chorologie der Zentraleuropäischen Flora. Band **II**. Tekst & Karten. Fischer Verlag, Jena.

Menken, S.B.J. (1981) Host races and sympatric speciation in small ermine moths, Yponomeutidae. Entomol. exp. appl. **30**, 280-292.

Moran, N.A. (1983) Seasonal shifts in host usage in *Uroleucon gravicorne* (Homoptera: Aphididae) and implications for the evolution of host alternation in aphids. Ecol. Entomol. **8**, 371-382.

Moran, N.A. (1986) Morphological adaptation to host plants in *Uroleucon* (Homoptera: Aphididae). Evolution **40**, 1044-1050.

Moran, N.A. (1987) Evolutionary determinants of host specifity in *Uroleucon*. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 96-99. SPB Academic Publishing, The Hague.

Moran, N.A. (1988) The evolution of host-plant alternation in aphids: evidence for specialization as a dead end. Am. Nat. **132**, 681-706.

Moran, N.A. (1989) A 48-million-year-old aphid-host plant association and complex life cycle: biogeographic evidence. Science **125**, 173-175.

Mordvilko, A. (1934) On the evolution of aphids. Arch. Naturgesch., N.F., **3**, 1-60.

Müller, F.P. (1971) Isolationmechanismen zwichen sympatrischen bionomischen Rassen am Beispiel der Erbsenblattlaus *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae). Zool. Jb. Abt. Syst. Oecol. Geogr. Tiere **98**, 131-152.

Müller, F.P. (1980) Wirtspflantzen, Generationsfolge und reproductive Isolation intraspecifiker Formen von *Acyrthosiphon pisum*. Entomol. exp. appl. **28**, 145-157.

Müller, F.P. (1985) Genetic and evolutionary aspects of host choice in phytophagous insects, especially aphids. Biol. Zbl. **104**, 225-237.

Müller, F.P. (1986) The role of subspecies in aphids for applied entomology. J. appl. Ent. **101**, 295-303.

Müller, F.P. & Hubert-Dahl, M.L. (1973) Wirtspflantzen und Überwinterung eines an Erbse lebenden Biotyps von *Acyrthosiphon pisum* (Harris). Dt. ent. Z., N.F., **20**, 321-328.

Müller, F.P. & Hubert-Dahl, M.L. (1979) Wirtswechsel, Generationsfolge und reproduktive Isolation von *Ovatus crataegarius* (Walker, 1850) und *O. insitus* (Walker, 1850) (Homoptera: Aphididae). Dt. ent. Z., N.F. **26**, 241-253.

Narzikulov, M.H. & Daniarova, M.M. (1979) [A new species of aphididae of the genus *Cryptomyzus* Oestl., 1922 (Homoptera: Aphididae) from Tadzhikistan], in Russian. Trudy Vses. ent. Obshch. **61**, 42-44.

Prokopy, R.J., Bennett, E.W. & Bush, G.L. (1971) Mating behavior in *Rhagoletis pomonella* (Diptera: Tephritidae). I. Site of assembly. Can. Ent. **103**, 1405-1409.

Remaudière, G. & Davitchi, A. (1961) Un *Cryptomyzus* (Hom.; Aphidoidea) nouveau de l'Iran. Rev. Path. vég. Ent. agric. Fr. **15**, 3-10.

Smith, D.C. (1988) Heritable divergence of *Rhagoletis pomonella* host races by seasonal asynchrony. Nature **366**, 66-67.

Shaposhnikov, G. Ch. (1966) Origin and breakdown of reproductive isolation and the criterion of the species. Entomol. Rev. **45**, 1-18.

Shaposhnikov, G. Ch. (1985) The main features of evolution in aphids. In: Evolution and Biosystematics of aphids. Proceedings of the International Aphidological Symposium at Jablona, Poland, 5-11 April, 1981. pp. 19-99. Ossolineum.

Stroyan, H.L.G. (1958) Contribution to the taxonomy of some British species of *Sappaphis* Matsumura 1918 (Homoptera, Aphidoidea). J. Linn. Soc. **43**, 644-713.

Stroyan, H.G.L. (1985) Recent developments in the taxonomic status of the genus *Dysaphis*

Börner. In: Evolution and Biosystematics of aphids. Proceedings of the International Aphidological Symposium at Jablona, Poland, 5-11 April, 1981. pp. 347-392. Ossolineum.

Thompson, J.N. (1988) Evolutionary genetics of oviposition preference in swallowtail butterflies. Evolution **42**, 1223-1234.

Thompson, J.N. (1989) Concepts of coevolution. TREE **4**, 179-183.

Via, S. (1986) Genetic covariance between oviposition preference and larval performance in an insect herbivore. Evolution **40**, 778-785.

Visser, J.H. & Taanman, J.W. (1987) Odour-conditioned anemotaxis of apterous aphids (*Cryptomyzus korschelti*) in response to host plants. Physiol. Entomol. **12**, 473-479.

von Dohlen, C.D. (in press) Evolutionary loss of the secondary host in heteroecious aphids: phylogenetic evidence. In: Proceedings of the III. International Symposium on Aphids, 1989, Kecskemet, Hungary.

von Dohlen, C.D. & Gill, D.E. (1989) Geographic variation and evolution in the life cycle of the witch-hazel leaf gall aphid, *Hormaphis hamamelidis*. Oecologia **78**, 165-175.

Ward, S.A. (1987) Cyclical parthenogenesis and the evolution of host range in aphids. In: Population structure, genetics and taxonomy of aphids and Thysanoptera. (ed. by J. Holman, J. Pelikán, A.F.G. Dixon and L. Weismann), pp. 39-44. SPB Academic Publishing, The Hague.

Ward, S.A. (in press) Sympatric speciation in aphids. In: Proceedings of the III. International Symposium on Aphids, 1989, Kecskemet, Hungary.

Zwölfer, H. & Herbst, J. (1988) Präadaptation, Wirtskreiserweiterung und Parallel-Cladogenese in der Evolution von phytophagen Insekten. Z. zool. Syst. Evolut.-forsch. **26**, 320-340.

APPENDIX. LIST OF SAMPLING LOCALITIES OF *Cryptomyzus*.

Locality, in ( ) province abbreviation, or country if not the Netherlands; sample date; morphs; host plant. * marked samples used for electrophoretic study.
Province: D=Drente, F=Friesland, G=Gelderland, L=Limburg, NB=Noord-Brabant, NH=Noord-Holland, U=Utrecht.
B=Belgium, BRD=Bundes Republiek Deutschland, CSSR=Czechoslovakia, F=France, GB=Great Britain, H=Hungary, S=Sweden.
Abbreviations of plant genera: B=*Ballota*, G=*Galeopsis*, L=*Lamium*, R=*Ribes*, S=*Stachys*.
morphs: F=fundatrix; apt=apterous viviparae; al=alate viviparae; ov=oviparae; M=male; la=larvae

C. galeopsidis (Kaltenbach)

Rhenen(U)	23.09.83	apt	*G. tetrahit*
Wageningen(G)	03.05.84	F	*R. nigrum*
Wageningen(G)	07.05.84	F	*R. nigrum*
* Rhenen(U)	18.05.84	apt, al	*R. nigrum*
* Randwijk(G)	28.05.84	apt	*R. nigrum*
* Randwijk(G)	28.05.84	apt	*R. rubrum*
* Wageningen(G)	03.07.84	apt	*R. rubrum*
* Wageningen(G)	03.07.84	apt	*R. nigrum*
* Rhenen(U)	11.07.84	apt, al	*Galeopsis* sp.
* Rhenen(U)	11.07.84	apt	*R. nigrum*
* Rhenen(U)	11.07.84	apt	*R. rubrum*
* Wageningen(G)	13.07.84	apt, al	*G. tetrahit*
* Nigtevecht(U)	18.07.84	apt	*R. nigrum*
* Nigtevecht(U)	18.07.84	apt	*G. tetrahit*
* Wageningen(G)	23.07.84	apt	*R. rubrum*
* Wageningen(G)	25.07.84	apt	*Galeopsis* sp.
* Wageningen(G)	25.07.84	apt	*R. rubrum*
* Wageningen(G)	31.07.84	apt	*G. segetum*
* Wageningen(G)	01.06.84	apt	*G. tetrahit*
* Suameer(F)	06.08.84	apt, al	*G. tetrahit*
* Suameer(F)	06.08.84	apt, al	*G. bifida*?
* Wageningen(G)	08.08.84	apt	*R. nigrum*
* St Geertruid(L)	09.08.84	apt	*G. tetrahit*
* St Geertruid(L)	09.08.84	al	*L. galeobdolon*
* Rhenen(U)	15.08.84	apt	*G. tetrahit*
* Wageningen(G)	20.08.84	apt, al	*G. tetrahit*
* Hemmen(G)	21.08.84	apt	*R. rubrum*
Randwijk(G)	21.08.84	apt	*R. rubrum*
Wageningen(G)	17.09.84	apt	*R. rubrum*
* Wageningen(G)	27.09.84	apt, al	*R. rubrum*
Rhenen(U)	03.05.85	F	*R. nigrum*
Rhenen(U)	03.05.85	F	*R. rubrum*
* Wageningen(G)	15.05.85	apt, al	*R. rubrum*
* Bennekom(G)	06.06.85	apt, al	*G. tetrahit*
* Wageningen(G)	06.06.85	apt	*R. nigrum*
* Wageningen(G)	07.06.85	apt	*R. nigrum*
Rhenen(U)	07.06.85	apt	*G. tetrahit*
* Rhenen(U)	07.06.85	al	*R. rubrum*
* Wageningen(G)	11.06.85	apt	*L. amplexicaule*
* Wageningen(G)	25.07.85	apt, al	*R. rubrum*
* Wageningen(G)	25.07.85	apt	*R. nigrum*

sample localities

* Rhenen(U)	25.07.85	apt, al	*R. nigrum*
* Rhenen(U)	25.07.85	apt, al	*R. rubrum*
* Rhenen(U)	25.07.85	apt	*G. tetrahit*
* Wageningen(G)	01.08.85	apt, al	*G. speciosa*
* Wageningen(G)	06.08.85	apt	*R. rubrum*
* Bennekom(G)	14.08.85	apt	*G. tetrahit*
* Wageningen(G)	19.08.85	apt	*R. rubrum*
* Maastricht(L)	25.09.85	apt, al	*G. tetrahit*
* Rhenen(U)	26.09.85	apt, al, la, M	*G. tetrahit*
* Wageningen(G)	24.10.85	ov	*R. rubrum*
Wageningen(G)	13.05.86	apt	*R. nigrum*
Wageningen(G)	13.05.86	apt	*R. rubrum*
Wageningen(G)	14.05.86	apt	*G. tetrahit*
Rhenen(U)	14.05.86	apt	*R. nigrum*
Wageningen(G)	26.05.86	la	*R. rubrum*
Wageningen(G)	26.05.86	la	*R. rubrum*
Wageningen(G)	26.05.86	la	*R. rubrum*
Wageningen(G)	04.06.86	la	*R. rubrum*
Nigtevecht(NH)	15.06.86	la	*R. nigrum*
Nigtevecht(NH)	15.06.88	la	*R. rubrum*
Wageningen(G)	25.06.86	apt	*R. rubrum*
Wageningen(G)	03.07.86	apt	*G. tetrahit*
* Wageningen(G)	13.08.86	apt, al	*L. amplexicaule*
* Wageningen(G)	13.08.86	apt	*L. purpureum*
Wageningen(G)	13.08.86	apt	*R. rubrum*
Amsterdam(NH)	14.08.86	apt	*R. nigrum*
Amsterdam(NH)	14.08.86	apt	*G. tetrahit*
Amsterdam(NH)	14.08.86	apt	*R. rubrum*
* Bunde-Elsloo(L)	20.08.86	apt	*G. tetrahit*
* Meerssen(L)	20.08.86	apt	*R. rubrum*
Wageningen(G)	25.08.86	apt	*L. purpureum*
Wageningen	28.08.86	apt	*R. rubrum*
* Wageningen(G)	01.09.86	apt, al	*Fragaria* cultivar
* Opheusden(G)	02.09.86	apt	*R. rubrum*
Nigtevecht(NH)	07.09.86	apt	*G. tetrahit*
* Beilen(D)	10.09.86	apt	*G. tetrahit*
Wageningen(G)	18.09.86	apt	*R. rubrum*
* Rhenen(U)	01.10.86	apt, al	*R. nigrum*
* Rhenen(U)	01.10.86	apt, al	*L. purpureum*
Wageningen(G)	01.10.86	apt,al, ov	*R. rubrum*
Wageningen(G)	15.10.86	al, ov, M	*R. rubrum*
* Rhenen(U)	23.04.87	F	*R. nigrum*
Rhenen(U)	23.04.87	F	*R. rubrum*
Lienden(G)	27.04.87	la	*R. rubrum*
* Bennekom(G)	28.04.87	F	*R. nigrum*
* Workum(F)	03.05.87	F	*R. nigrum*
Lienden(G)	04.05.87	F	*R. rubrum*
* Amsterdam(NH)	05.05.87	F, la, al	*R. nigrum*
* Amsterdam(NH)	05.05.87	F, apt, la, al	*R. nigrum*
Amsterdam(NH)	05.05.87	F, apt, la, al	*R. rubrum*

sample localities

Wageningen(G)	05.87	apt	*R. rubrum*
* Driebergen(U)	07.05.87	apt, la, al	*R. nigrum*
* Santpoort(NH)	14.05.87	F, apt, la, al	*R. nigrum*
Santpoort(NH)	14.05.87	F, apt, la, al	*R. rubrum*
* Zuiderwoude(NH)	05.06.87	apt, la, al	*R. nigrum*
Wageningen(G)	03.05.88	F	*R. nigrum*
Bennekom(G)	04.05.88	F	*R. nigrum*
Rhenen(U)	04.05.88	F	*R. nigrum*
Zuiderwoude(NH)	06.05.88	F, apt	*R. nigrum*
Ede(G)	09.05.88	F, apt	*R. nigrum*
Wageningen(G)	01.08.88	apt	*R. nigrum*
Zuiderwoude(NH)	02.10.88	apt, al, la, M	*R. nigrum*
* Riede, Niedersachsen, BRD	09.82	apt	*G. tetrahit*
* Tübingen, Baden Württemberg, BRD	06.83	apt	*G. tetrahit*
* Tübingen, Baden Württemberg, BRD	06.84	apt	*G. tetrahit*
* Riede, Niedersachsen, BRD	08.84	apt	*B. nigra*
* Brezova, Moldavie, CSSR	14.09.85	apt, al	*G. tetrahit*
* Prague, Bohemia, CSSR	16.09.85	la, ov	*R. rubrum*
St. Etienne de Boulogne, Ardèche, F	14.07.86	apt	*G. segetum*?
* Börrigesjön, Skåne, S	05.10.86	al	*G. tetrahit*
* Lund, Skåne, S	07.10.86	apt	*L. hybridum*
Hollóstetö, Bükk, H	26.08.87	apt	*G. tetrahit*
Regéc, Tokaj, H	29.08.87	apt	*G. speciosa*
Tolcva, Tokaj, H	29.08.87	apt	*G. tetrahit*
Yell, Shetland, GB	20.07.88	apt	*R. nigrum*

C. ribis (L.)

* Wageningen(G)	07.82	apt	*R. sanguineum*
Wageningen(G)	25.04.84	apt	*R. rubrum*
* Opheusden(G)	02.05.84	apt	*R. rubrum*
* Lienden(G)	02.05.84	apt	*R. rubrum*
* Opheusden(G)	02.05.84	apt	*R. rubrum*
* Lienden(G)	03.05.84	apt	*R. nigrum***uva-crispa*
* Lienden(G)	03.05.84	apt	*R. rubrum*
* Lienden(G)	03.05.84	apt	*R. nigrum*
* Wageningen(G)	03.05.84	apt	*R. rubrum*
* Bennekom(G)	10.05.84	F	*R. rubrum*
* Renkum(G)	11.05.84	F	*R. rubrum*
* Wageningen(G)	14.05.84	F	*R. rubrum*
* Randwijk(G)	28.05.84	apt	*R. nigrum*
* Randwijk(G)	28.05.84	apt	*R. rubrum*
* Randwijk(G)	28.05.84	apt	*R. rubrum*
* Wageningen(G)	29.05.84	apt	*R. rubrum*

sample localities

* Eindhoven(NB)	25.06.84	apt	*R. rubrum*
* Wageningen(G)	03.07.84	apt	*L. amplexicaule*
* Nigtevecht(NH)	18.07.84	apt	*R. rubrum*
* Nigtevecht(NH)	18.07.84	apt	*S. palustris*
* Wageningen(G)	25.07.84	?	*L. maculatum*
* Wageningen(G)	31.07.84	apt	*G. segetum*
* Suameer(F)	06.08.84	apt, la, al	*S. palustris*
Plasmolen(L)	19.09.84	apt, la, al	*S. palustris*
* Bennekom(G)	06.06.85	apt, la, al	*R. rubrum*
* Wageningen(G)	01.08.85	apt	*G. speciosa*
Rhenen(U)	13.08.85	apt	*S. palustris*
* Wageningen(G)	24.10.85	ov	*R. rubrum*
Wageningen(G)	13.05.86	F	*R. rubrum*
Wageningen(G)	25.06.86	apt, al	*R. rubrum*
* Wageningen(G)	13.08.86	apt	*L. amplexicaule*
* Wageningen(G)	13.08.86	apt	*L. purpureum*
* Wageningen(G)	13.08.86	apt	*R. rubrum*
* Geulle(L)	20.08.86	apt	*S. palustris*
* Elsloo(L)	20.08.86	apt	*S. sylvatica*
* Wageningen(G)	28.08.86	apt	*S. annua*
* Wageningen(G)	01.09.86	apt	*Leonurus cardiaca*
* Wijster(D)	09.09.86	apt, la, al	*S. palustris*
* Wageningen(G)	18.09.86	apt	*S. officinalis*
Amsterdam(NH)	05.05.87	F	*R. rubrum*
Driebergen(U)	07.05.87	F	*R. nigrum*
Wageningen(G)	07.05.87	la	*R. sanguineum*
* Wachendorf, Baden Württemberg, BRD	06.83	?	*R. nigrum*
* Riede, Niedersachsen, BRD	05.84	?	*R. rubrum*
* Tübingen, Baden Württemberg, BRD	08.86	?	*Stachys*
* Falsterbo, Skåne, S	06.10.86	la, al	*S. palustris*
Tübingen, Baden Württemberg, BRD	05.07.87	la	*Leonurus cardiaca*

C. alboapicalis (Theobald)

Bennekom(G)	23.09.83	apt	*L. album*
* Weesp(NH)	18.07.84	apt, la, al	*L. album*
* Nigtevecht(U)	18.07.84	apt	*L. album*
Rhenen(U)	31.07.84	apt	*L. album*
Rhenen(U)	31.07.84	apt	*B. nigra*
* Rhenen(U)	01.08.84	apt	*L. album*
* Megen(NB)	02.08.84	apt	*L. album*
* Suameer(F)	06.08.84	apt	*L. album*

sample localities

St Geertruid(L)	09.08.84	la	*L. album*
* Rhenen(U)	15.08.84	apt, al	*L. album*
Megen(NB)	19.09.84	apt	*L. album*
Rhenen(U)	01.08.85	apt	*L. album*
* Wageningen(G)	06.08.85	apt	*L. album*
* Beusichem(G)	11.08.85	apt	*L. album*
* Achterberg(U)	13.08.85	apt	*L. album*
* Rhenen(U)	13.08.85	apt, al	*L. album*
* Rhenen(U)	01.10.86	apt	*L. purpureum*
* Riede, Niedersachsen, BRD	09.82	?	*L. album*
* Pumpernudl, Bayern, BRD	06.84	?	*L. album*
* Freiröttenbach, Bayern, BRD	07.84	?	*L. album*
* Honfleur, Cavados, F	19.11.84	apt	*L. album*
* Sindelfingen, Baden Württemberg, BRD	09.86	?	*L. album*
* Falsterbo, Skåne, S	03.10.86	apt	*L. album*
Norwich, East Anglia, GB	22.02.88	apt	*L. album*

C. alboapicalis*(L. maculatum) = *C. ulmeri (Börner)

* Wageningen(G)	25.07.84	apt	*L. maculatum*
* Rhenen(U)	03.05.85	apt, la, al	*L. maculatum*
* Rhenen(U)	07.06.85	apt, al	*L. maculatum*
* Rhenen(U)	07.06.85	apt, al	*L. maculatum*
* Wageningen(G)	06.08.85	apt	*L. maculatum*
* Wageningen(G)	06.08.85	apt	*L. maculatum*
* Geulle(L)	20.08.86	apt	*L. maculatum*
* Smolenice, Moldavië, CSSR	13.09,85	apt	*L. maculatum*
* Kanne, Limgurg, B	25.09.85	apt, la, M	*L. maculatum*
* Rudesheim, Hessen, BRD	12.10.86	ov, la, M	*L. maculutum*

C.korschelti Börner

* Wageningen(G)	07.82	?	*S. sylvatica*
Rhenen(U)	23.09.83	apt	*S. sylvatica*
* Bennekom(G)	13.07.84	apt	*R. alpinum*
* Wageningen(G)	13.07.84	apt, la, al	*R. alpinum*
* Bijlmermeer(NH)	18.07.84	apt	*S. sylvatica*
* Amsterdam(NH)	22.07.84	apt	*R. alpinum*
* Wageningen(G)	25.07.84	apt	*S. sylvatica*
* Rhenen(U)	01.08.84	apt	*S. sylvatica*

sample localities

St Geertruid(L)	09.08.84	la	*S. sylvatica*
* Bennekom(G)	06.06.85	apt	*R. alpinum*
* Wageningen(G)	06.08.85	apt,	*R. alpinum*
* Wageningen(G)	06.08.85	apt, al	*S. sylvatica*
* Maastricht(L)	25.09.85	apt, al	*S. sylvatica*
Wageningen	31.05.86	F	*R. alpinum*
* Bunde-Elsloo(L)	20.08.86	apt	*S. sylvatica*
Wageningen(G)	06.05.87	apt	*R. alpinum*
* Tübingen, Baden Württenberg, BRD	06.83	?	*S. sylvatica*
* Tübingen, Baden Württenberg, BRD	05.84	?	*R. alpinum*
* Honfleur, Calvados, F	19.11.84	apt	*S. sylvatica*
* Pietersberg, Limburg, B	25.09.85	apt, al	*S. sylvatica*
Tübingen, Baden Württenberg, BRD	05.07.87	apt	*S. sylvatica*
Hollóstetö, Bükk, H	26.08.87	apt	*S. sylvatica*
Sauveterre-la-Lémance, Lot-et-Garonne, F	13.09.87	apt	*S. sylvatica*

C.ballotae HRL

* Wageningen(G)	08.82	?	*B. nigra*
Rhenen(U)	11.08.84	apt, la, al	*B. nigra*
* Rhenen(U)	01.08.84	apt	*B. nigra*
* Megen(NB)	02.08.84	apt, la, al	*B. nigra*
* Rhenen(U)	14.08.84	apt	*L. album*
Megen(NB)	19.09.84	apt	*B. nigra*
* Rhenen(U)	13.08.85	apt	*B. nigra*
* Norwich, East Anglia, GB	04.04.85	apt	*B. nigra*
Norwich, East Anglia, GB	22.02.88	apt	*B. nigra*

C. galeopsidis*(L. galeobdolon) = *C. maudamanti **sp.n.** Guldemond

* St Geertruid(L)	09.08.84	apt	*L. galeobdolon*
* Hemmen(G)	21.08.84	apt	*L. galeobdolon*
Rhenen(U)	25.06.85	apt	*L. galeobdolon*
* Rhenen(U)	25.07.85	apt, la, al	*L. galeobdolon*
* Wageningen(G)	06.08.85	apt	*L. galeobdolon*
* Rhenen(U)	13.08.85	apt	*L. galeobdolon*
* Bennekom(G)	14.08.85	apt	*L. galeobdolon*

sample localities

* Rhenen(U)	26.09.85	apt, la, al	*L. galeobdolon*
* Bunde(L)	20.08.86	apt	*L. galeobdolon*
* Tübingen, Baden Württemberg, BRD	06.83	?	*L. galeobdolon*
* Tübingen, Baden Württemberg, BRD	06.84	?	*L. galeobdolon*
* Tübingen, Baden Württemberg, BRD	06.86	?	*L. galeobdolon*

C. heinzei HRL

* Tübingen, Baden Württemberg, BRD	05.84	?	*R. alpinum*
Hecho, Aragon, Spain	29.07.86	apt	*S. officinalis*
* Tübingen, Baden Württemberg, BRD	05.07.87	apt	*S. officinalis*
* Tübingen, Baden Württemberg, BRD	05.07.87	apt, la, al	*R. alpinum*
Repashuta, Bükk, H	28.08.87	apt	*S. officinalis*

C. (Ampullosiphon) stachydis (Heikinheimo)

* Janakkala, Finland	19.06.85	apt, al	*R. spicatum*