On the origin of uniparental cytoplasmic inheritance

The effects of heteroplasmy on the fitness of *Trametes versicolor*

(*Trametes versicolor*, curbstonevalley.com)

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Abstract

In obligate symbiotic relationships, a conflict may arise between symbiont and host over symbiont transmission, due to different selective pressures on the different partners in the symbiosis. A well-studied instance of such a symbiotic relationship is the eukaryotic cell and its mitochondria. It has been hypothesized that in almost all living species mitochondria are inherited uniparentally in order to minimize the potential for conflict among mitochondrial and between nucleic and mitochondrial genes. A fundamental problem with this hypothesis is that the prevention of conflict provides only a long-term, population-wide benefit, making it very difficult to be selected for on an individual level. In this project it was tested whether there also is a short-term advantage of preventing heteroplasmy (having two different mitochondrial strains in one cell). This was done using the Basidiomycete fungus *Trametes versicolor*. Growth-rates (as a proxy for fitness) were compared between homoplasmic and heteroplasmic dikaryons with identical nuclei. With a few exceptions, no differences in growth-rates between homoplasmic and heteroplasmic dikaryons were found. Furthermore, no evidence of segregation of mitochondria, indicated by a sectored growth style of the heteroplasmic dikaryons, was witnessed. Finally, a series of di-di and di-mon pairings did not provide any evidence for an influence of cytoplasmic factors on the formation or strength of inter-mycelial barrages. To conclude, at the very most only quite ambiguous evidence for a short-term negative effect of heteroplasmy on the fitness of *Trametes versicolor* was found.

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Introduction

Host-symbiont conflict

Countless organisms are known for having close symbiotic relationships with species from related or different genera, families, phyla, or even kingdoms. Some well-known examples include fig-wasps and figs (Frank 1996), microbes living in the human gut (Belzer & de Vos 2012), fungus-growing termites (Aanen *et al.* 2009), and soil-dwelling microbiota and plants (Schnitzer *et al.* 2011). As diverse as these examples may seem, they all potentially share a similar problem: a conflict may arise between host and symbiont over symbiont transmission, driven by natural selection (Frank 1996). In this project I studied one possible instance of such a conflict.

A host-symbiont conflict arises as a consequence of three factors (Frank 1996). First, due to the Hamilton and May (1977) effect, selection favours some symbionts to disperse from their host in order to avoid competition with closely-related individuals. Second, due to this dispersal symbiont-mixing will occur within hosts, decreasing the average relatedness between symbionts. At some point it will become more rewarding for symbionts to invest in traits amplifying within-host competition to increase their relative reproductive success, rather than in traits beneficial for the success of the whole host-symbiont community (Bremermann & Pickering 1983). Therefore, hosts favour limited symbiont-mixing and dispersal. These three factors together may potentially lead to host-symbiont conflict, as a result of selection working on different levels for the different partners in the symbiosis.

The potential for conflict is strongest in interactions with the following four characteristics (Frank 1996): firstly, the relationship between host and symbiont is obligate - symbionts live together with or within every host; secondly, symbionts may invest in traits increasing their own relative reproductive success, but decreasing the group and host fitness; thirdly, competition between symbionts is increased by higher symbiont mixing; and fourthly, hosts are able to control symbiont dispersal and mixing.

Mitochondria

Mitochondria meet all these four criteria, and are perfect examples of the potential for host-symbiont conflict (Eberhard 1980, Aanen *et al.* 2014). They are obligate in (almost) all eukaryotic cells, generating the cell's ATP-supply. They are capable of within-cell competition (Hintz *et al.*1988, Taylor *et al.* 2002) and hostile take-over of mitochondria from a different host (Lee & Taylor 1993, Fischer & Seefelder 1995, Fischer & Wolfrath 1997, Yan & Xu 2003), possibly endangering the host's energy production. And finally, hosts have plenty of possibilities to limit mitochondrial dispersal and mixing (Hurst & Hamilton 1992, Frank 1996). These characteristics set the stage for potential nucleo-mitochondrial conflict.

There are plenty of examples of the detrimental effects mitochondria may have on their host's fitness because of this conflict. In both plants (Budar *et al.* 2003) and fungi (Aanen *et al.* 2004), so-called selfish mitochondria may increase their reproductive success at the cost of their male hosts by causing cytoplasmic male sterility (CMS). Mutations that have a negative effect on male fitness but a (near) neutral effect on female fitness, a phenomenon known as the Mother's Curse (Gemmel *et al.* 2004), lead to male ageing in *Drosophila* and a wide range of diseases in humans (Frank & Hurst 1996, but see Beekman *et al*. 2014). A final example shows that horizontally transmitted cancer in dogs may live long past its normal life-span, when it is supplied with new, functional mitochondria in new hosts - a process which is hugely detrimental for the dogs, but beneficial for

the mitochondria (Aanen & Maas 2012).

Many studies agree that this great potential for nucleo-mitochondrial conflict has given rise to the uniparental inheritance of mitochondria and other cytoplasmic elements (Frank *et al.* 1996). Uniparental inheritance will drastically decrease the presence of mixed cytoplasmic lineages, and therefore the potential for host-symbiont conflict. (However, interestingly enough some of the examples mentioned in the previous paragraph may have arisen as secondary problems of uniparental inheritance.) According to Hurst & Hamilton (1992), this selection for the minimization of the potential for conflict eventually even led to the evolution of binary mating systems, although recently developed theoretical models argue the benefit of uniparental inheritance alone is not enough to have driven the evolution of such a mating system (Hadjivasiliou *et al*. 2013). There are also two more fundamental problems with this explanation for the origin of uniparental mitochondrial inheritance.

First of all, there are many known instances of heteroplasmy (cells containing two different strains of mitochondria) and deviations from uniparental inheritance of mitochondria in animals, plants, fungi, and other organisms (Barr *et al.* 2005, Ni *et al.* 2011, Wilson & Xu 2012). For example, in the blue mussel *Mytilus*, females inherit mitochondrial DNA (mtDNA) only from their mother, whereas males inherit it from both parents (Zouros *et al.* 1994). Interestingly enough, heteroplasmy does not necessarily seem to have a negative effect on host fitness, as is seen in organisms such as (but not limited to) the slime mold *Physarum polycephalum* (Sakurai *et al.* 2004), the fungus *Coprinus cinereus* (Baptista-Ferreira *et al*. 1983), and even in humans, where hereditary heteroplasmy in leukocytes seems to occur more in centenarians (Rose *et al.* 2007), although longevity does not necessarily equate to higher fitness.

Secondly, even though reduced mitochondrial mixing will eventually lead to decreased within-host competition, the positive effects of this on host fitness will only become clear after several generations for the whole host population, not immediately for individual hosts (Hoekstra 1987). Other studies also suggest uniparental inheritance mainly has a long-term benefit: Bastiaans and colleagues (2014) show that selection for dysfunctional mtDNA variants within cells of *Neurospora* is prevented by high within-cell relatedness, and the model developed by Hadjivasiliou and colleagues (2012) indicates mitonuclear co-adaptation (Wolff *et al*. 2014) is also limited by heteroplasmy. This raises the question whether there may be a short term positive effect of uniparental inheritance for the host as well.

Fungi

Fungi are the perfect system for testing hypotheses about the possible short-term negative effects of heteroplasmy. This is because of the unique system of sexual reproduction in (Basidiomycete) fungi: two monokaryons (haploid mycelia, each containing a single nucleus per cell), will exchange nuclei with each other, forming one dikaryon, with two different nuclei per cell (Nieuwenhuis 2012, see figure 1). Cytoplasm is not exchanged, except for a small contact-zone between the two original monokaryons, where a barrage is formed (Day 1959, Baptista-Ferreira *et al.* 1983, Hintz *et al.* 1988). Therefore, the newly-created dikaryon forms a cytoplasmic mosaic: some parts contain mitochondria from one parent, some parts from the other parent, and the small contact-zone is heteroplasmic (Aanen *et al.* 2004).

This occurrence of heteroplasmy as part of the normal life cycle of fungi opens up the opportunity for heteroplasmic organs and individuals and mitochondrial recombination, as has been shown in many species, such as *Coprinus lagopus* (Day 1959), *Coprinus cinereus* (Baptista-Ferreira *et al.*

1983), *Agaricus bitorquis* (Hintz *et al.* 1988), *Agaricus bisporus* (Xu *et al.* 2013), *Saccharomyces cerevisiae* (Zinn *et al.* 1987, Strausberg & Perlman 1978, Taylor *et al.* 2002), *Cryptococcus neoformans* (Yan *et al.* 2004, Yan *et al.* 2007), *Armillaria* (Smith *et al.* 1990, Saville *et al.* 1998), *Neurospora* (Yang & Griffiths 1993), *Neurospora tetrasperma* (Lee & Taylor 1993), *Pleurotus ostreatus* and *P. pulmonarius* (Fischer & Seefelder 1995, Fischer & Wolfrath 1997), and many others (Wilson & Xu 2012). Furthermore, in a wide range of species it has been shown that considerable intraspecific mitochondrial DNA variation exists: in *Beauveria bassiana* (Uribe & Khachatourians 2004), *Mycosphaerella graminicola* (Torriani *et al*. 2008), *Candida albicans* (Bartelli *et al*. 2013), *Rhizophagus irregularis* (Formey *et al*. 2012, Beaudet *et al*. 2013, de la Providencia *et al.* 2013), and *Glomus intraradices* (Börstler *et al.* 2008).

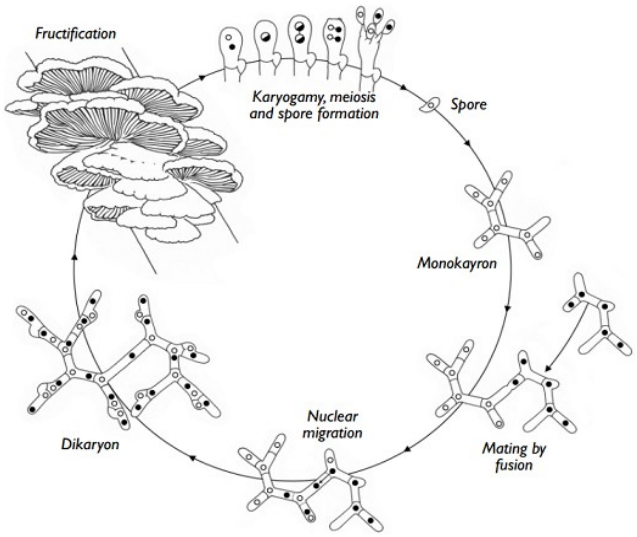

Figure 1. Life cycle of *Schizophyllum commune*, a typical Basidiomycete. Note the nuclear exchange and migration, represented by the small white and black circles (the two different nuclei). Figure originally from Nieuwenhuis (2012).

However, it has to be noted that heteroplasmy is usually only observed in the lab in a few cells and is often followed by quick segregation of the different mitochondrial strains (Day 1959, Baptista-Ferreira *et al.* 1983, Zinn *et al.* 1987, Hintz *et al.* 1988, Lee & Taylor 1993, Yan & Xu 2003, Yann *et al.* 2007, de la Providencia *et al.* 2013). One possible explanation as to how this segregation of mitochondrial DNA occurs is that one of the variants has a replication advantage (Aanen *et al*. 2014), which may be acquired by genome size reduction (Selosse *et al*. 2001). No matter the mechanism, could this quick segregation of mitochondrial variants mean there is a short-term negative effect of heteroplasmy after all?

A very strong indication for this comes from a study performed by Sharpley and colleagues in 2012. By mixing two normal but different mouse mtDNAs, they created a line of heteroplasmic mice. It was found that these heteroplasmic mice, but neither of their homoplasmic parent lines, had reduced activity and food intake, cognitive impairment, a lowered respiratory exchange ratio, and an accentuated stress response. The exact mechanism through which this occurs is unknown – the authors suggest a reduced efficiency of oxidative phosphorylation or perturbed cellular signalling between the mitochondrion and the nuclear genome, but differences in reactive oyxgen-species and mtDNA copy number have also been implicated (Lane 2012) – yet it is very clear that heteroplasmic mice have a lower fitness than their homoplasmic relatives.

Hypotheses

The main goal of this project is to test whether induced heteroplasmy in the Basidiomycete fungus *Trametes versicolor* has a similar effect on its fitness. To test this, the following hypotheses were formulated:

1) Heteroplasmic dikaryons have lower growth-rates than homoplasmic dikaryons.
This is to be expected based on theory (Frank 1996) and the observed practice of quick segregation of mitochondria (Day 1959, Hintz *et al.* 1988, Yan *et al.* 2007) and hostile take-over of mitochondria from a different host (Lee & Taylor 1993, Fischer & Seefelder 1995, Fischer & Wolfrath 1997, Yan & Xu 2003). Mycelial growth-rate is chosen as a proxy for the fitness of the fungus (Pringle & Taylor 2002, Schoustra *et al.* 2012).

2) Some heteroplasmons will be characterised by a sectored growth style.
In at least some cases, one would expect that the different strains of mitochondria segregate, causing homoplasmic sectors within a heteroplasmic dikaryon (Day 1959, Hintz *et al.* 1988).

3) Barrage formation can be predicted based on (dis)similarities in cytoplasmic background rather than nuclei.
Antagonism between mycelia is characterised by the formation of a barrage (Rayner & Todd 1977, Williams *et al*. 1981). If such antagonism is an effect of conflict between mitochondria, its occurrence can be predicted based on the cytoplasmic backgrounds of the respective mycelia involved in a pairing.

These hypotheses are based on the following assumptions:

1) Sampling cells from the contact-zone of two monokaryons creates a heteroplasmic dikaryon (Day 1959, Zinn *et al.* 1987, Hintz *et al.* 1988, Smith *et al.* 1990).
2) When segregation of mitochondrial strains occurs, it is clearly visible, because of the appearance of sectors (Day 1959, Hintz *et al.* 1988).

Materials and Methods

Collection and preparation of the strains

In the fall of 2012 and winter of 2014 several fruiting bodies of *Trametes versicolor* were collected in the forests around Wageningen and Dieren, The Netherlands. Small sections of the caps of these fruiting bodies were pasted to the lid of a Petri dish, with the gills pointing down to the malt yeast agar (MYA) medium in the dish. After being stored for a day in a warm and humid environment, in most Petri dishes several small colonies, originating from spores fallen down from the caps, were growing. For each individual fruiting body one small colony, corresponding to a single spore-origin, was inoculated onto a new Petri dish. In addition to this, several strains originating from the UK and the USA were kindly supplied by professor Lynne Boddy of the Cardiff School of Biosciences. This resulted in a total collection of 9 monokaryotic strains. These strains were stored at 24 degrees Celsius and used in the further experiments.

Growth experiment

For (almost) all possible combinations, two monokaryons, belonging to the same species, were inoculated onto a new Petri dish with MYA medium. After storing them for several days by 24 degrees Celsius to allow mycelial growth and dikaryonization to take place, small sections of a few square millimetres were cut out from the newly formed dikaryon and transferred to new dishes. Per dikaryon, six sections were cut out: two from the barrage which formed where the two monokaryons met, and two for each parent, from the newly-formed dikaryotic ridges flanking the barrage (see figure 2).

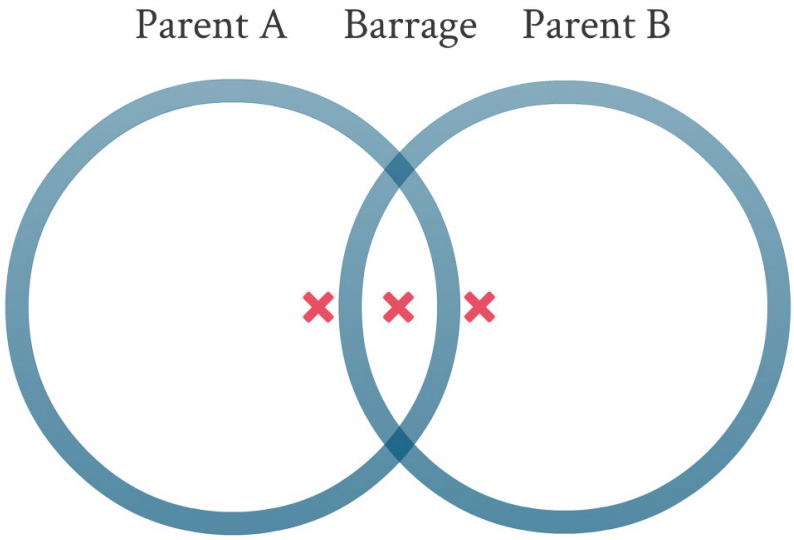

Figure 2. Schematic overview of the sampled sections of the dikaryons used for the growth experiment. The blue circles represent the mycelial growth of both the parent monokaryons, the red crosses denote the sampling locations (please note that each cross was sampled *in duplo*, resulting in six sections per dikaryon.)

Mycelial growth-rates were measured and compared between the different sections of the same dikaryon in two different sessions: first for twenty-one unique combinations of strains and subsequently for six different combinations. After several days of growth, it was tested whether each of these sections were truly dikaryonized, by checking for the presence of clamp-connections

which indicate the occurrence of nuclear migration and therefore dikaryonization (Nieuwenhuis 2012). As growth-rates of dikaryons tend to be higher than growth-rates of monokaryons (Swietzynski & Day 1960, Kües 2000), only truly dikaryonized sections were used in further analyses.

Di-mon pairings

To test whether the cytoplasmic background of a mycelium has any influence on the formation and size of a barrage, a series of di-mon pairings was performed. A specific dikaryon was paired with three monokaryons: once with a monokaryon containing a nucleus not present in the original dikaryon; once with a monokaryon containing a nucleus that is present in the dikaryon, but that has a different cytoplasm; and once with a monokaryon containing both the same nucleus and cytoplasm as the dikaryon. This experiment was performed with eight different dikaryons.

Di-di pairings

Finally, to test whether a difference in cytoplasmic background alone is enough to create a barrage, two dikaryons with identical nuclei but different cytoplasms were paired. This experiment was performed with four different dikaryons.

Results

Growth experiment

The vast majority of all inoculated sections turned out to be fully dikaryonized. Only six pairings, all from the first session, had to be excluded from further analyses due to a lack of clamp-connections. None of the presumed heteroplasmons had a sectored growth style. The results can be seen in figures 3 and 4 and tables 1 and 2. For the complete results, see the appendix.

Session 1	Day		
Average mycelial diameter	**3**	**4**	**5**
Parent A	3.11	5.29	7.12
Barrage	2.97	5.11	6.87
Parent B	3.27	5.40	7.13
Parent average	3.19	5.35	7.12

Table 1. Average mycelial diameters in centimetres for different sections after 3, 4, and 5 days of growth, respectively. Session 1.

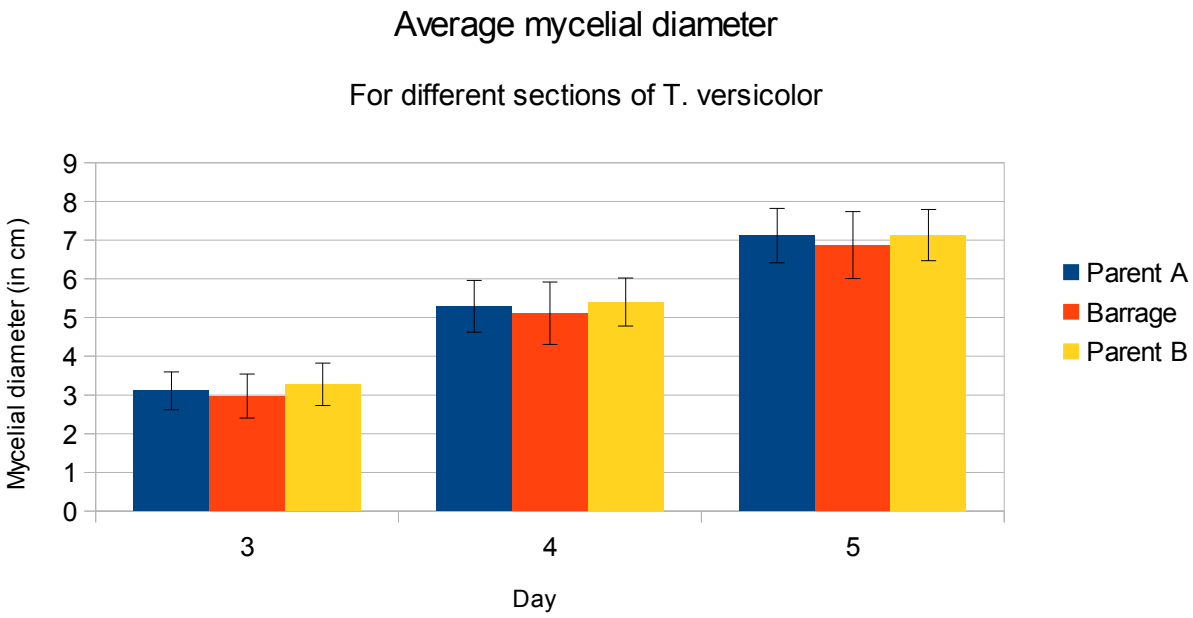

Figure 3. Average mycelial diameters in centimetres for different sections of dikaryons of *T. versicolor*, session 1. Error bars denote the respective standard deviations.

Session 2	Day	
Average mycelial diameter	**4**	**5**
Parent A	4.96	6.52
Barrage	4.61	6.26
Parent B	4.68	6.28
Parent average	4.82	6.4

Table 2. Average mycelial diameters in centimetres for different sections after 4 and 5 days of growth, respectively. Session 2.

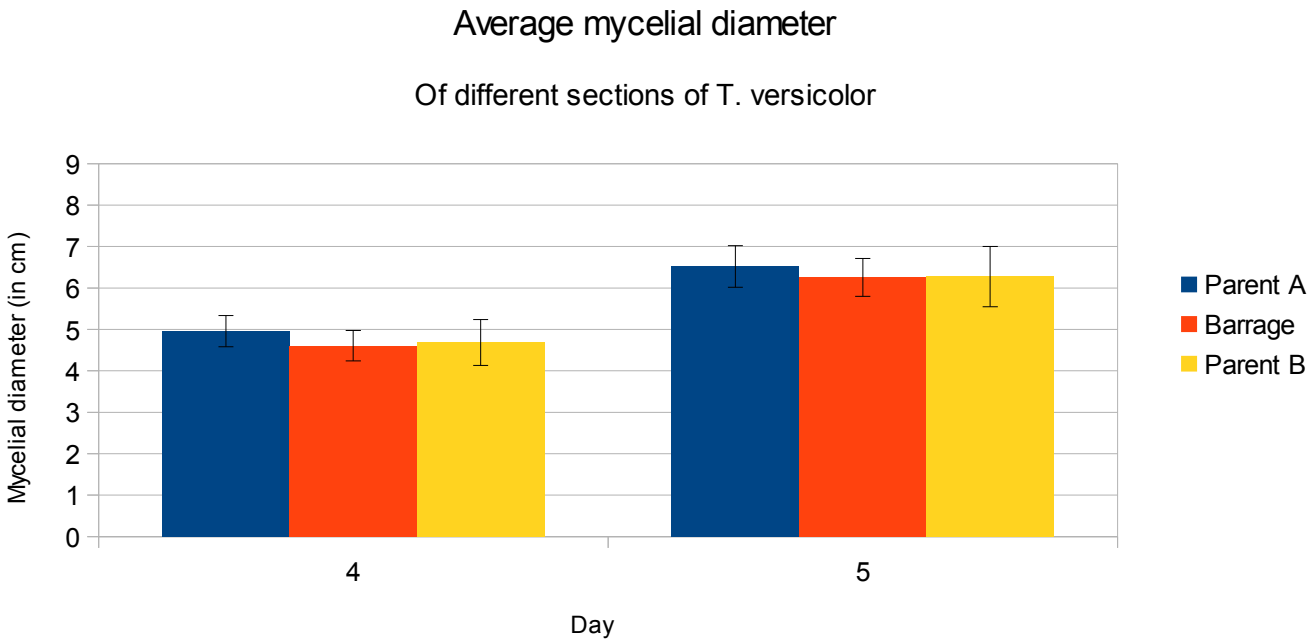

Figure 4. Average mycelial diameters in centimetres for different sections, session 2.

To test whether any apparent differences in mycelial growth-rates are statistically significant, a two-tailed Student's t-test for paired samples (Moore *et al*. 2009) was performed. For the results, see tables 3 and 4. A significance level of 0.05 is used.

Session 1	**Day**		
P-value for paired samples t-test	**3**	**4**	**5**
Parent A – barrage	0.154	0.067	0.009*
Parent B – barrage	0.001*	0.004*	0.009*
Parent average – barrage	0.011*	0.009*	0.004*
Parent A – Parent B	0.035*	0.185	0.879

Table 3. P-values obtained using a Student's t-test for paired samples to test whether differences in average growth-rates between different sections are statistically significant, session 1. * = statistically significant.

Session 2	**Day**	
P-value for paired samples t-test	**4**	**5**
Parent A – barrage	0.024*	0.090
Parent B – barrage	0.585	0.892
Parent average – barrage	0.058	0.163
Parent A – Parent B	0.146	0.214

Table 4. P-values obtained using a Student's t-test for paired samples to test whether differences in average growth-rates between different sections are statistically significant, session 1. * = statistically significant.

The results from the first session of the growth experiment deserve closer scrutiny. Before the experiment was performed, the pairings were divided in two groups, based on a visual assessment of the strength of their barrage. Pairings with a clearly visible barrage and two dikaryotic ridges were placed in group one, all the other pairings in group two. The first group consisted of four samples, the second group of eleven. In figures and tables 5 and 6 the results of the growth experiment averaged per group are shown.

Group 1	Day		
Average mycelial diameter	**3**	**4**	**5**
Parent A	3.53	5.66	7.38
Barrage	2.96	5.01	6.78
Parent B	3.65	5.72	7.35
Parent average	3.59	5.69	7.37

Table 5. Average mycelial diameters in centimetres for different sections of pairings with a clearly present barrage.

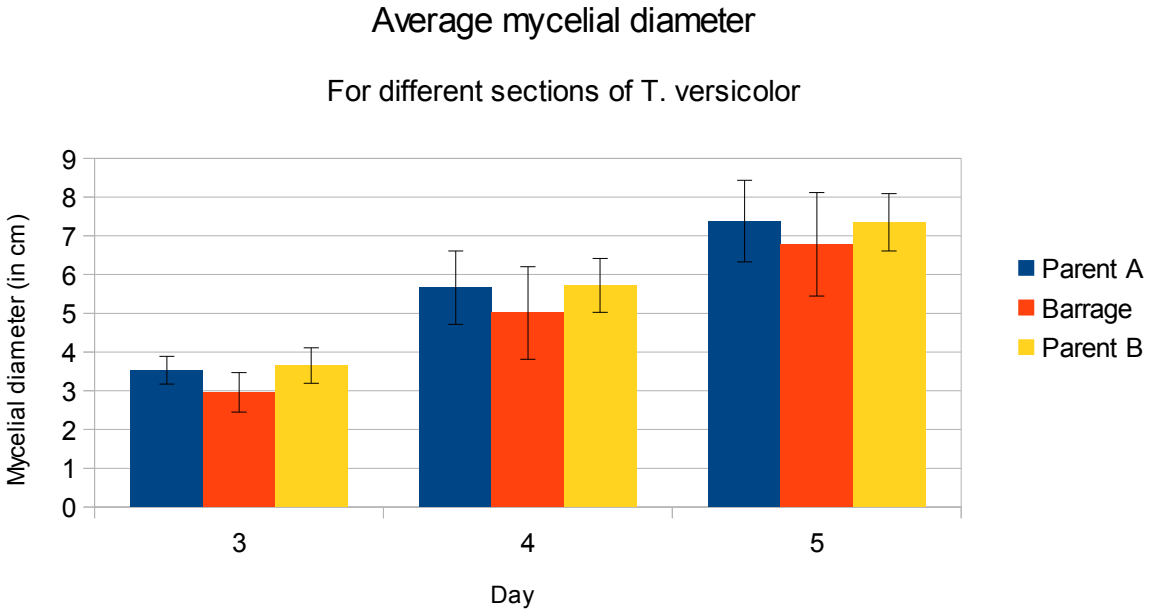

Figure 5. Average mycelial diameters in centimetres for different sections of pairings with a clearly present barrage.

Group 2	Day		
Average mycelial diameter	**3**	**4**	**5**
Parent A	2.92	5.12	7.00
Barrage	2.98	5.15	6.91
Parent B	3.10	5.25	7.03
Parent average	3.01	5.19	7.01

Table 6. Average mycelial diameters in centimetres for different sections of pairings with a less-clearly present barrage.

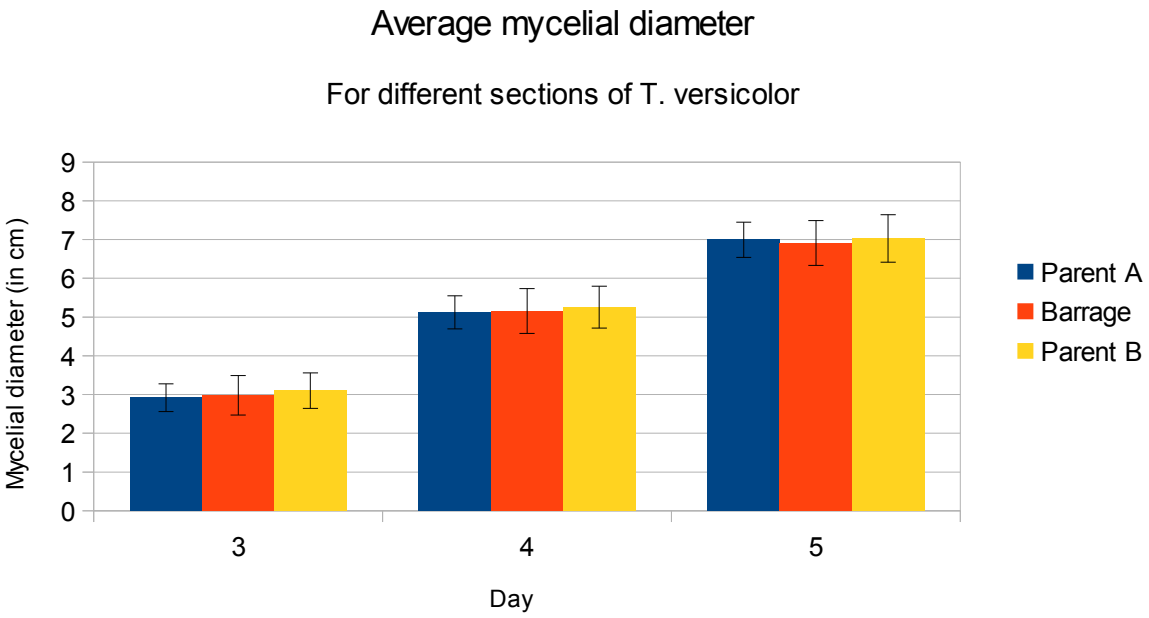

Figure 6. Average mycelial diameters in centimetres for different sections of pairings with a less-clearly present barrage.

Again, the growth-rates were analyses using a Student's t-test. See tables 7 and 8.

Group 1	**Day**		
P-value for paired samples t-test	**3**	**4**	**5**
Parent A – barrage	0.002*	0.001*	0.003*
Parent B – barrage	0.000*	0.004*	0.023*
Parent average – barrage	0.000*	0.002*	0.007*
Parent A – Parent B	0.168	0.614	0.827

Table 7. P-values obtained using a Student's t-test to test whether differences in average growth-rates between different sections derived from pairings with clearly visible barrages are statistically significant. * = statistically significant.

Group 2	**Day**		
P-value for paired samples t-test	**3**	**4**	**5**
Parent A – barrage	0.551	0.737	0.369
Parent B – barrage	0.190	0.220	0.182
Parent average – barrage	0.692	0.624	0.180
Parent A – Parent B	0.089	0.229	0.761

Table 8. P-values obtained using a Student's t-test to test whether differences in average growth-rates between different sections derived from pairings with less-clearly visible barrages are statistically significant. * = statistically significant.

Finally, it was tested whether found differences in growth-rates remained significantly different after the colonies were inoculated onto a new Petri dish. The results are shown in figure 7 and tables 9 and 10.

		Day		
Average mycelial diameter		**3**	**4**	**5**
	Parent A	4.35	6.00	7.33
	Barrage	4.43	6.13	7.55
	Parent B	4.30	6.08	7.60
	Parent average	4.33	6.04	7.46

Table 9. Average mycelial diameters in centimetres for different sections after additional inoculation.

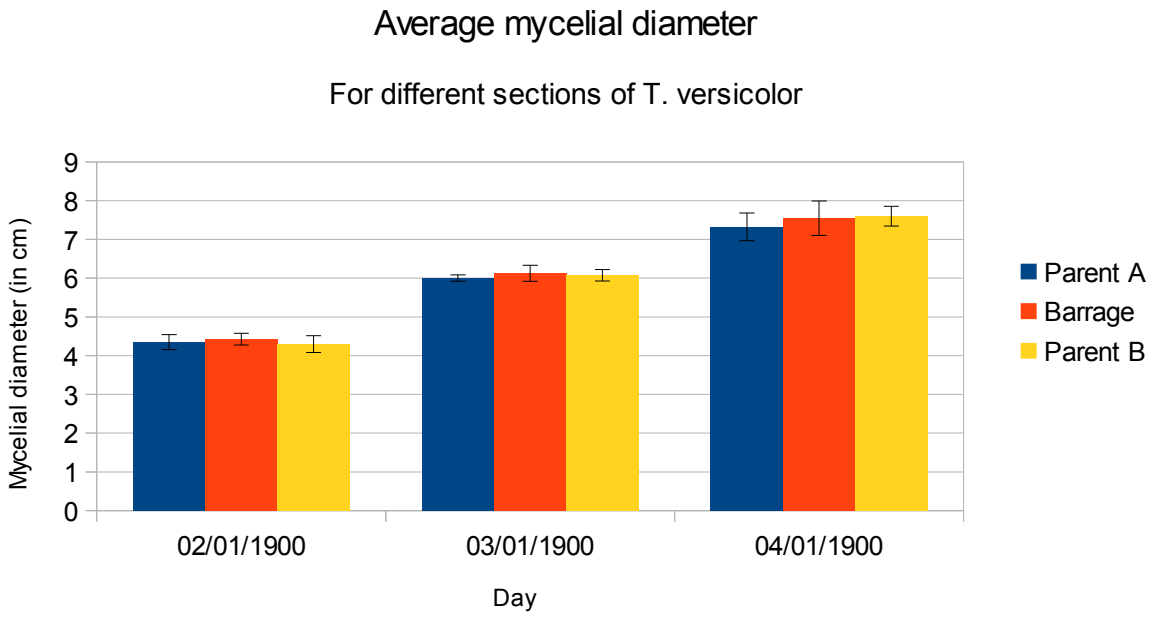

Figure 7. Average mycelial diameters in centimetres for different sections after additional inoculation.

		Day		
P-value for paired samples t-test		**3**	**4**	**5**
	Parent A – barrage	0.547	0.312	0.384
	Parent B – barrage	0.492	0.604	0.718
	Parent average – barrage	0.468	0.379	0.633
	Parent A – Parent B	0.731	0.444	0.151

Table 10. P-values obtained using a Student's t-test to test whether differences in average growth-rates between different sections are statistically significant after additional inoculation. * = statistically significant.

Di-mon and di-di pairings

In none of the di-mon pairings a substantial difference was found in either formation or size of the barrage between the different monokaryons. No barrages were formed in the di-di pairings.

Discussion

The first hypothesis formulated in this report states that heteroplasmic dikaryons have lower growth-rates than homoplasmic dikaryons. If the mycelia I used were in fact heteroplasmic (which may be disputed, see discussion below), the results of the growth experiment are at the very least ambiguous. As can be seen in figure 3, the growth rates of fifteen heteroplasmic dikaryons was lower than that of their homoplasmic 'parents', and table 3 shows this difference is statistically significant. This result clearly agrees with the hypothesis mentioned above. Unfortunately, this effect could not be replicated in a second experiment with six new combinations of strains.

Furthermore, on closer scrutiny, the results of the first experiment are not as convincing as they seem. The fifteen dikaryons used in this experiment could be divided into two groups: one group, consisting of four pairings, was characterised by a clearly present barrage, and one group, consisting of eleven pairings, was characterised by a faint or partially absent barrage. As it turns out, the significant results described above were solely caused by the four pairings with a very clear barrage (see figures 5 and 6).

Therefore the only support for the hypothesis that heteroplasmy lowers fitness in *T. versicolor* comes from the results of four dikaryons, out of a total sample of twenty-one. Finally, after two (out of four) of these presumably heteroplasmic dikaryons with lower growth-rates were inoculated onto a new Petri dish, the difference in growth-rates disappeared altogether (see figure 7). This could either mean that segregation of mitochondria happens relatively quickly (Yan *et al*. 2007), causing the negative effects of heteroplasmy to disappear within a few days, or that some other effect explains the lack of growth of cells sampled from the barrage. One explanation could be that the barrage is generally characterised by a much lower cell density than the surrounding areas (Rayner & Todd 1977, Williams *et al*. 1981).

All of these results taken together fail to provide strong support for the first hypothesis. Support for the second hypothesis, which sets forth that some heteroplasmons will be characterised by a sectored growth style, is even weaker. In none of the experiments a mycelium with a sectored growth style was found. The third hypothesis, which states that barrage formation can be predicted based on (dis)similarities in cytoplasmic background rather than nuclei, is not supported by the results either. Both formation and strength of the barrage were shown to be independent of cytoplasmic background in a series of eight di-mon pairings. Similarly, in four pairings between dikaryons with identical nuclei but different cytoplasms, no barrages were formed.

Do these results indicate that, unlike in mice (Sharpley *et al*. 2012), there is no negative effect of heteroplasmy in *T. versicolor*? I would argue that it may be a bit too early to reject the hypotheses yet. The main reason for this is that the whole interpretation of this project is completely based on its inherent assumptions. If assumption one is true, and sampling from the barrage area of two monokaryons does create a heteroplasmic dikaryon, then it seems reasonable to assume that heteroplasmy at the most only has a faint negative effect on fitness, at least in *T. versicolor.* However, that assumption has not been tested in this project. Therefore, it is not known whether there was in fact heteroplasmy, or that the different mitochondrial strains have long been segregated (Hintz *et al*. 1988, Lee & Taylor 1993, Yan *et al.* 2007). This means that it is not clear what has been tested in this project: the effect of heteroplasmy, of nuclear imprinting, of cell density, or of unknown factors? Based on the results from this project alone it is not possible to distinguish between these explanations.

Furthermore, much remains unclear about the exact process that underlies the formation of the

barrage. In a sample of twenty-one, only four pairings were found with a strong and clearly visible barrage. Mycelia sampled from these barrages were also the only (presumably) heteroplasmic mycelia with lower growth-rates. This could indicate that only in a limited number of cases the antagonistic effect between mitochondria is strong enough to create a clear barrage and a negative effect on mycelial fitness. A possible explanation is that such an antagonism may only occur when two mitochondrial strains have sufficiently different genomes; it may therefore be no coincidence that two out of these four pairings consisted of one sample from the United Kingdom and one from the Netherlands, increasing the likelihood of mitochondrial genomic variation (Wolff *et al*. 2014). On the other hand, of the remaining seventeen pairings, four were formed with a combination of parents from the United Kingdom and the Netherlands as well, and no lower growth-rates were measured in those samples. This question will hopefully be answered satisfactory in the near future, as the mitochondrial genomes of several samples are in the process of being sequenced and analysed, which will give full insight in the level of intraspecific genomic variation.

A different explanation for the results observed in this project is that the heteroplasmic phase in *T. versicolor* is extremely transient, only existing in a short time window (Day 1959, Yann *et al.* 2007, de la Providencia *et al.* 2013). Adding to that, it is my personal observation that the clarity and strength of barrages seem to alter subtly over time. Finding the right moment for the sampling of the barrage – after it has been formed, but before segregation takes place – might therefore be crucial for experiments like the ones performed in this report. Finally, it was noted by Renes & van Veen (2014) that the strength of a barrage may not only depend on time, but also on the medium on which the samples were inoculated. Using a different medium than MYA, especially for the di-mon and di-di pairings, may therefore yield different results.

Taking everything in consideration, I can only conclude that this project at the very most provides extremely limited support for the hypothesis that heteroplasmy has a direct negative fitness effect in *Trametes versicolor.* Results from analyses that will be finished in the near future, such as the sequencing of mtDNA and a protoplasting experiment, may change this conclusion, and the considerations mentioned above should be kept in mind. but at the moment I would argue that it is not entirely unreasonable to reject the hypotheses formulated in the introduction.

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Appendix

Growth experiment, session 1

Code	Left	Middle	Right	
3-UK		4	3.3	3.9
		4	3.4	4
3-13		3.5	2.9	3.3
		3.5	2.8	3.7
3-15		3.9	3.7	4.5
		3.5	3.8	4
UK-15		3.7	3	3.8
		3.8	3.1	3.8
3-8		2.9	3.1	3.5
		3.3	3.3	3.9
3-12		2.3	1.7	1.9
		3.1	2	2
3-14		3.5	3.8	3
		3	2.9	3.8
8-UK		3.3	3.4	3.3
		3	3.2	3.4
8-12		2.5	2.7	3
		2.8	2.7	3
8-14		2.8	2.8	2.9
		2.7	2.5	3.1
8-15		3.1	3.2	3.2
		3.3	3.1	3.3
UK-14		2	3	3
		3	3	3.1
12-14		2.5	3.3	3.3
		3.2	3.3	3.4
13-14		3	2.7	3.1
		2.7	2.8	3.1
14-15		3.1	4	2.9
		3.1	3	3
01/04/14				
3-UK		6.4	5.8	6.1
		6.3	5.9	6.3
3-13		6	5.1	5.4
		5.9	4.9	5.9
3-15		6.3	6	6.4
		5.9	6	6
UK-15		6	5.1	6.1
		6	5.5	6
3-8		5	5.4	6
		5.8	6	6.3
3-12		4.3	3.5	4
		5.3	4.3	4.1
3-14		5.4	6	5.5
		5.2	5.4	5.8
8-UK		5.7	5.4	5.1
		4.9	5.1	5.5

Group 1

Group 2

01/04/14	Left	Middle	Right	
8-12		4.3	4.9	4.9
		4.8	4.5	5
8-14		4.8	5.1	5
		5.2	4.8	5
8-15		5.6	5.4	5.4
		5.7	5.5	5.8
UK-14		5	5.1	4.8
		5	5	5.2
12-14		5.1	5.5	5.6
		5.5	5.5	5.6
13-14		5.2	4.9	5.4
		4.4	4.8	5.3
14-15		5.3	6	5.2
		5.2	5.3	5.1
02/04/14				
3-UK		8	7.5	7.7
		8	7.4	7.9
3-13		8	6.9	7.3
		7.8	7	7.5
3-15		7.9	7.9	8
		7.9	7.8	7.5
UK-15		7.6	7.2	7.7
		7.8	7.4	7.9
3-8		7.5	7.4	7.9
		7.8	7.8	8
3-12		6.4	5.5	6
		7.2	5.9	6.1
3-14		7.1	7.6	6.9
		6.8	7	7.4
8-UK		7.5	7.3	7.2
		6.8	7.1	7.5
8-12		6.2	6.5	6.5
		6.4	6.1	6.4
8-14		6.6	7	6.9
		7	6.3	6.8
8-15		7.6	7.3	7.5
		7.6	7.3	8
UK-14		6.8	6.9	6
		7	6.8	6.8
12-14		6.8	7.3	7.7
		7.4	7.4	7.5
13-14		6.7	6.7	7
		6.4	6.5	6.8
14-15		7.3	7.3	6.8
		7	7	6.9

Growth experiment, session 2

22/04/14	Left	Middle	Right	
10-12		5	4.1	4.7
		4.3	4.7	4.3
9-15		4.3	4.6	5.1
		5	4.7	5
3-9		5.3	4.7	5
		4.9	4.8	5
UK-10		5.7	5	5
		5.1	5	5.4
UK-9		5	4.6	4.8
		5	4.6	4.6
10-14		4.9	3.7	3.6
		5	4.8	3.7
23/04/14				
10-12		6.5	6.2	6.1
		5.5	6	5.8
9-15		6.1	6.5	7
		6.9	6.5	7
3-9		6.8	6.3	6.7
		6.3	6.7	6.6
UK-10		7.4	6.8	6.7
		7	6.7	7
UK-9		6.5	6.2	6.2
		6.8	6.1	6.3
10-14		6.2	5.1	4.9
		6.2	6	5

Session 1, growth after re-inoculation

14/04/14	Left	Middle	Right	
3-UK		4.1	4.3	4.4
		4.3	4.6	4
3-13		4.5	4.5	4.5
		4.5	4.3	4.3
15/04/14		4.35	4.425	4.3
3-UK		5.9	5.9	6
		6	6.3	6
3-13		6	6.3	6.3
		6.1	6	6
16/04/14				
3-UK		7.4	7.9	7.9
		7.8	7.7	7.7
3-13		7	7.7	7.5
		7.1	6.9	7.3