Geographic variation in male mate choice in a gynogenetic species complex: evaluating long-term data across mating contexts

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ABSTRACT

Aim: To consider how geographic variation in species recognition over time affects the speciation process.

Organisms: The gynogenetic livebearing fish Poecilia formosa (Amazon molly) is the hybrid offspring of two bisexual parent species, P. latipinna (sailfin molly) and P. mexicana (Atlantic Molly). The Amazon molly is an all-female species that must mate with males of their parent species. But the males of the parent species gain no genetic benefit from these matings, so the Amazon mollies are sexual parasites. Poecilia latipinna males prefer to mate with conspecific females in most sympatric populations but show varying levels of mating mistakes across their broad geographic distribution.

Questions: How do reproductive isolation barriers change over time and across populations of the parent species that have been allopatric to, or sympatric with, gynogens for varying amounts of time? Why do some individual populations show consistent species recognition while others do not?

Methods: We reviewed the literature and summarized the data from populations for which species recognition had been examined multiple times. We also used our 15-year long data set. We examined variation in species recognition by mollies both temporally (on an evolutionary time scale) and geographically.

Conclusions: Species recognition by male sailfin mollies was more consistent in allopatric and early sympatric conditions compared with male sailfin mollies in populations that have existed longer in sympathy with Amazon mollies. However, sailfin molly populations that have co-existed for longer do have more isolation barriers to mating.

Keywords: Poecilia formosa, Poecilia latipinna, reproductive isolation, speciation, temporal variation.
INTRODUCTION

Environments vary in abiotic characteristics, as well as in biotic interactions. Local adaptations may be found when populations live in patchy environments and are subject to different selective pressures (Robinson and Schluter, 2000). Local adaptation may ultimately lead to reproductive isolation, and variation across populations in species interactions can cause variation in selection on mating traits (Hoskin and Higge, 2010). Exploring geographic variation in the mechanisms by which animals select mates, as well as the ultimate fitness benefits of expressing mating biases is necessary to understand the processes that drive speciation.

It is important to explore both geographic variation and temporal variation in mating behaviours across populations, as abiotic and biotic contexts are likely to vary (Endler, 1977; Endler and Houde, 1995; Lynch et al., 2005; Heubel and Schlupp, 2008; Lehtonen et al., 2010; Kahn et al., 2013). Only examining mate choice in a few populations is not likely to provide insights into the mating preference of the whole species. Understanding geographic and temporal variation in mating behaviours can also provide insight into the process of speciation, especially when populations are sympatric with closely related species. Moreover, biotic and abiotic conditions can change through time on an ecological time scale. Indeed, Pfennig (2007) showed that female spadefoot toads, Spea bombifrons, encounter temporal variation (year to year) in pond longevity and switch from choosing conspecifics to heterospecifics under certain pond conditions.

Mate choice may also be affected by the length of time that two closely related species have co-existed (evolutionary time scale). Over time, multiple reproductive barriers may arise that further promote speciation (Nosil et al., 2005; Butlin et al., 2012). By examining which barriers to heterospecific matings might arise over time, it may be possible to determine the mechanisms required for the speciation process to complete. Furthermore, by examining mate choice in the same populations across multiple years (ecological time scale), it may be possible to determine whether barriers change, or become more complex, as well as whether there is decay in reproductive isolation (Butlin et al., 2012).

STUDY SYSTEM AND PAST WORK

A good system for evaluating how geographic and temporal variation in species interactions may drive behavioural variation is a gynogenetic species complex consisting of several livebearing (Family: Poeciliidae) fish species: the unisexual gynogenetic Poecilia formosa (Amazon molly) and its bisexual parent species P. latipinna (sailfin molly) and P. mexicana (Atlantic molly) (Hubbs and Hubbs, 1932; Kallman, 1962; Darnell et al., 1967). Amazon mollies putatively arose from a hybridization event between a female P. mexicana limantouri and a male P. latipinna (or another extinct sailfin species) ~100,000 years ago [200,000 generations (Avise et al., 1991; Schartl et al., 1995; Lampert and Schartl, 2008; Stöck et al., 2010; Alberici da Barbiano et al., 2013)]. Amazon mollies are gynogenetic, meaning they require insemination from a parent (host) species to start embryogenesis; however, inheritance is strictly maternal and, as a result, males gain no known paternity benefits (but see Schlupp et al., 1994) from mating with these sexual parasites. In this unisexual–bisexual mating complex, male recognition errors are essential for the reproduction and maintenance of the gynogenetic species, yet are costly for males of the sexual species (Aspbury and Gabor, 2004b; Schlupp and Plath, 2005).

Amazon mollies are found from Rio Tuxpan in Mexico and northward to the Nueces River in Texas along the coast of the Gulf of Mexico (Darnell and Abramoff, 1968; Miller, 1983; reviewed...
The sailfin molly is widely distributed along the Gulf of Mexico from the Atlantic coast of North Carolina south to the Rio Tuxpan in Mexico (Miller, 1983). Atlantic mollies are found from Rio San Fernando, Mexico south to Honduras and possibly beyond (Miller, 1983). Poeciliids invaded Central and North America from the south (Hrbek et al., 2007), suggesting that sailfin and Atlantic mollies might have colonized populations moving northward. The centre of origin of the Amazon molly is likely in the region of Tampico in northern Mexico (Avise et al., 1991; Schartl et al., 1995), and they may have slowly expanded their range northward and southward. In addition, Amazon mollies have been introduced into, and have established populations, in several rivers in central Texas (Brown, 1953).

The distribution of these three species provides a great scenario for examining geographic variation in species recognition by males of the parent species, given that there is variation in populations in species interactions (sympatric with or allopatric to Amazon mollies), as well as variation in the length of time of co-existence in sympatric populations. Populations of the parent species further north and south of the origin of Amazon mollies have co-existed with Amazon mollies for less time than those populations closer to the area of origin in Tampico, Mexico. Given that populations are widespread and likely have been

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**Fig. 1.** A map of the distribution of sailfin mollies (*Poecilia latipinna*), Atlantic mollies (*P. mexicana*), and Amazon mollies (*P. formosa*). The light grey area is the distribution of sailfin mollies, the hatched area is the distribution of Atlantic mollies, and the solid black outline is the distribution of Amazon mollies. Included are the localities of the six populations discussed in this paper and in the tables.
syntopic with Amazon mollies for varying amounts of time, we predict that males from
different populations of the parent species will differ in their species recognition. Indeed,
males of both parent species prefer to mate with conspecific females in most sympatric
populations (Hubbs, 1964; Ryan et al., 1996; Gabor and Ryan, 2001; Schlupp and Plath, 2005),
although less so for *P. mexicana* (Ryan et al., 1996; Gabor et al., 2012, and unpublished data).
On a finer scale, males from populations recently sympatric with Amazon mollies will differ in their species recognition
and consistency compared with males from populations that have co-existed for longer.
Short-term evidence for this is provided by Heubel and Schlupp (2008), where they do
not find a strong general preference by male sailfin mollies for conspecific females over
Amazon mollies in natural and introduced populations found in Texas. However, there
is also evidence for seasonal plasticity in male mate preferences in sailfin mollies
(Heubel and Schlupp, 2008).

Mate choice consists of both the identification of conspecific individuals (requiring
species recognition), as well as the selection of high-quality mates (requiring mate-quality
recognition) (Pfennig, 1998). Different selection regimes on mate choice can arise, as males in
sympatry generally face a conflict between species and mate quality recognition cues
(Pfennig, 1998; Gumm and Gabor, 2005). Here we review and synthesize data from studies on the proximate
mechanisms that might underlie species recognition from long-term studies of geographic
variation using several measures of mate choice, including mate preference, association
preference, sperm priming and expenditure, and sex steroid hormone production. Butlin
et al. (2012) suggested that a greater understanding of the process of speciation might arise by
documentation of the contribution that different reproductive barriers provide towards
effective reproductive isolation in multiple populations across a range of overall isolation
levels. The populations of mollies that we have been studying vary in the amount of time
in sympathy across their geographic range, and there is also variation across time and space
in the frequency of Amazon mollies. By evaluating the diverse mechanisms by which males
base mate-choice decisions, we hope to learn more about the role of variation in behavioural
and physiological responses to potential mates. Based on 15 years of data, we examine the
degree of consistency or variability in male mate choice within and between populations
and across metrics of assessing mate preference. This information will enhance our
predictions about how male species recognition and discrimination might have ultimate
effects on the fitness of individuals of interacting species, as well as inform our predictions
about the co-existence of a gynogen with its sexual host.

**EVALUATING MATE CHOICE**

There are different methods to evaluate male mate choice in livebearing fish. Males can be
tested in either no-choice or dichotomous choice designs, and each of these methods allows
us to infer different aspects of the male mate preference process. Specifically, no-choice
designs allow for the evaluation of male mating permissiveness (the propensity to engage
in recognition errors), whereas dichotomous choice designs allow for the examination of
male mating discrimination between females of two species (see Alberici da Barbiano et al., 2012).
Generally, examining male mate discrimination based on association time (mate preference)
involves using a dichotomous choice design where the time an individual spends near one or
the other side of a testing tank is recorded, where females of each species are placed in
separated sections found at the extremities of the testing tank. Experiments aimed at
addressing questions about discrimination based on mate choice involve allowing the male
to interact freely with both females at the same time, and recording the number of mating attempts directed to either female.

Whereas dichotomous choice designs allow researchers to ask questions about mate preference and mate choice, they do not provide any information about the male’s propensity to make recognition errors (permissiveness). Male permissiveness can be tested if males are given the choice between one female on one side and nothing on the other side of the tank. This design does not allow males to compare females and, therefore, the male’s behaviour will rely only on the cues emitted by a single female. Depending on the question and the type of communication used by the species, visual cues, chemical cues or both cues simultaneously can be provided, depending on the set-up of the tank. With this design it is possible to examine which female cues are required by males to engage in species recognition and discrimination.

Male poeciliids have modified anal fins that function as intromittent organs, called gonopodia. To transfer sperm, males must thrust their gonopodium towards the female gonopore and, if they successfully connect, they may transfer sperm packets (spermatogonial units). Therefore, the most direct measure of male mate choice is to count the number of gonopodial thrusts males direct at a given female (using either dichotomous choice designs or no-choice designs).

In addition to examining behavioural variation in male mate choice and permissiveness, the two methods described allow us to examine the physiological responses of males when in the presence of conspecific and heterospecific females. One physiological response that we have focused on is the sperm priming response. Sperm priming is the increase in sperm production that occurs when males are given access to stimuli from females (either visual or chemical in the case of poeciliids). When sperm is released in bundles (spermatogonial units), these can be extracted from males and unpacked. The number of sperm cells produced after an association event can then be measured. The underlying assumption that justifies using priming response as a proxy for inferring mate preference is that spermatogenesis is energetically costly and can limit a male’s reproductive success (Nakatsuru and Kramer, 1982; Shapiro et al., 1994). Furthermore, variation in sperm production could be affected by pre-copulatory events such as mate choice, and may subsequently affect post-copulatory events such as conspecific sperm precedence (Aspbury and Gabor, 2004).

Another metric that can clarify the behavioural responses of males is hormonal response to mating with females. Sex steroid hormones can affect both male and female mating behaviour in fish (for reviews, see Hirschenhauser et al., 2004; Gabor and Grober, 2010; see also Gabor et al., 2012). Androgens may also affect the sperm priming response, as 11-ketotestosterone (KT) stimulates spermatogenesis in livebearing fish (Schreibman et al., 1986). Information about steroid hormones can be obtained by using either dichotomous choice or no-choice designs, depending on the particular question of interest.

**SUMMARY OF PATTERNS RELATED TO SPECIES RECOGNITION**

When tested in dichotomous choice experiments, both male sailfin and Atlantic mollies show species discrimination as they mate more with conspecific females (Ryan et al., 1996; Gabor and Ryan, 2001; Gumm and Gabor, 2005; Gabor and Aspbury, 2008; Aspbury et al., 2010) (Table 1; populations are identified in Fig. 1). In field experiments where males of both species are presented with both conspecific females and Amazon mollies, males of both species show species discrimination because they prefer to mate with conspecific females. 

Table 1. Summary of studies testing male mate preference in male sailfin mollies and Atlantic mollies in the same populations

<table>
<thead>
<tr>
<th>Study</th>
<th>Sailfin molly</th>
<th>Atlantic molly</th>
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<tbody>
<tr>
<td></td>
<td>Allopatry 1 (SC)</td>
<td>Sympatry 1 (AB)</td>
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<td></td>
<td>Introduced/recent sympatry (SP)</td>
<td>Sympatry 2 (VG)</td>
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<tr>
<td>Ryan et al. (1996)</td>
<td>Conspecific</td>
<td>Conspecific</td>
</tr>
<tr>
<td>Gabor and Ryan (2001)</td>
<td>Marginal preference</td>
<td>Conspecific</td>
</tr>
<tr>
<td>Aspbury and Gabor (2004b)</td>
<td>Conspecific</td>
<td></td>
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<tr>
<td>Gumm et al. (2006)</td>
<td>Conspecific</td>
<td></td>
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<tr>
<td>Gabor and Aspbury (2008)</td>
<td>Polymorphic conspecific</td>
<td>Polymorphic conspecific</td>
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<tr>
<td>Robinson et al. (2008)</td>
<td>Conspecific</td>
<td>Conspecific</td>
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<tr>
<td>Aspbury et al. (2010a)</td>
<td>Conspecific</td>
<td></td>
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<tr>
<td>Aspbury et al. (2010b)</td>
<td>Conspecific</td>
<td>No preference</td>
</tr>
<tr>
<td>Alberici da Barbiano et al.</td>
<td>Conspecific</td>
<td></td>
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<tr>
<td>(2010)</td>
<td></td>
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<tr>
<td>Gabor et al. (2012)</td>
<td>No preference</td>
<td></td>
</tr>
<tr>
<td>Gabor et al. (in preparation)</td>
<td>No KT response</td>
<td>No KT response</td>
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</tbody>
</table>

| SPECIES RECOGNITION           | NOT CONSISTENT | CONSISTENT | MOSTLY CONSISTENT | MOSTLY CONSISTENT | NOT CONSISTENT |

Note: Inclusion of studies performed in other laboratories was hindered by the lack of sampling coordinates in those studies. Regular font = experiments examining male thrusts; bold font = experiments examining male association preference; grey font = experiments examining hormone response; italic font = experiments examining male sperm priming.
Evidence from studies with a no-choice design suggests that males of the two species differ in their species discrimination but not permissiveness in species recognition. However, the permissiveness of male sailfin mollies appears to be dependent on the method used to assess mate preference. Male sailfin mollies from sympatric populations are not permissive, as they mate more with conspecifics than Amazon mollies when tested sequentially (Robinson et al., 2008; Gabor and Grober, 2010; J. Muraco et al., submitted) (Table 1). Aspbury and Gabor (2004b) also show that male sailfin mollies are not permissive because they prime more sperm when exposed to conspecific females than when exposed to heterospecific females. Male sailfin mollies also increase the rate of sperm production when in the presence of conspecific females but not when exposed to heterospecific females (Robinson et al., 2008). In addition, male sailfin mollies from an allopatric population are not permissive and prefer to associate with conspecific cues over a blank but not heterospecific cues over a blank, whereas males from a sympatric population do not show these preferences (Aspbury et al., 2010b). However, Alberici da Barbiano et al. (2012) tested male sailfin and Atlantic mollies and confirmed part of the results of Aspbury et al. (2010b), but found that males of both species were permissive in species recognition. In contrast to male sailfin mollies, male Atlantic mollies do not show
species discrimination, as they neither prime more sperm for conspecific over heterospecific females nor do they show sequential conspecific mating preference (Gabor et al., 2012). These results indicate that there is more variation in the discrimination of Atlantic mollies when tested with conspecific females and Amazons, but mostly not so for sailfin mollies in sympathy. It is important to note that both species are permissive in some scenarios.

Multiple cues in different modalities that signal the same or similar information are sometimes more effective at eliciting a response than just one cue (Rowe, 1999). When given access to only chemical cues, male sailfin mollies from a population sympatric with Amazon mollies are permissive, as they do not prime sperm differently when exposed to either conspecific or heterospecific cues (Aspbury et al., 2010b) (Table 1). In addition, male sailfin mollies lose their species discrimination between conspecific females and Amazon mollies if they are not given multiple cues, suggesting that their species discrimination and recognition processes generally require multiple sensory modalities. For example, male sailfin mollies do not discriminate between conspecific and heterospecific females if they are only given chemical cues (Aspbury et al., 2010b) or both cues from size-matched females but no tactile interactions (Gabor and Aspbury, 2008). However, when female sailfin mollies are larger or males only have access to visual cues from a transparency model, they show species discrimination in their association preference and prefer conspecific females (Gumm et al., 2006; Gabor and Aspbury, 2008). Yet, no isolated visual trait (fin size, body shape, spotting pattern) triggers species discrimination (Gumm et al., 2006), suggesting that male sailfin mollies require multiple cues concurrently during the species discrimination process.

Mate choice is likely also mediated by sex hormones. Gabor and Grober (2010) found that male sailfin mollies, from one population, show an increase in 11-ketotestosterone (KT) when mating with conspecifics but not when mating with heterospecifics (Table 1). Direct interaction with the females and multiple cues are likely necessary for the males to be successful in both the recognition process (the lack of permissiveness) as well as in the discrimination process. However, no increase in KT was detected in Atlantic mollies, from one population, when they mated with conspecifics (Gabor et al., 2012), suggesting, once more, that the males of the two parent species differ in their recognition and discrimination mechanisms even when given multiple cues and direct contact with the females. Nevertheless, hormones affect male mating behaviour in Atlantic mollies, as males that mated more with conspecific females had lower post-mating testosterone concentrations. These male Atlantic mollies also attempted to mate more often with conspecific females with lower post-mating estradiol concentrations (Gabor et al., 2012).

Another method to test variation in male mate preferences is to examine the repeatability (r, the intraclass correlation coefficient), or consistency of mating preferences. Repeatability is the relative degree of variation in mating preferences both within and between individuals. Understanding whether individuals are consistent in their mating behaviours is difficult to determine from values of r, because it is computed as a ratio. Similar values of r can indicate either consistent mate choice expressed by individuals (low variation between and within females) or random mate choice (high variation between and within females) (Widemo and Sæther, 1999). Consistency is estimated by the coefficient of variation in the strength of preference for a species, measured more than once. This is the variation in mating behaviours relative to each male’s successive values, and is independent of the variation across all other males (Cummings and Mollaghan, 2006).

Male sailfin mollies tested in a dichotomous mate choice design for association preference for larger conspecifics versus smaller heterospecifics are consistent with their mate choice
However, male mate choice in sailfin mollies showed overall low measures of repeatability in these experiments (Gabor and Aspbury, 2008). Gabor and Aspbury (2008) infer that individual males usually prefer conspecific females but some males consistently do not prefer conspecifics. This suggests that there may be individual variation in male mate preferences. However, it has been shown that male sailfin mollies prefer to mate with conspecifics but that their behavioural syndrome does not correlate with their mating preference (J. Muraco et al., submitted). The lack of a correlation between behavioural types and male mate choice in sailfin mollies may explain the lack of repeatability in male mate discrimination. These results suggest that discrimination and recognition errors are population wide and not limited to certain individuals in the population. Selection to mate with conspecifics may be counterbalanced by the limitations of changing behaviours across contexts, as is generally found when individuals of a species have behavioural syndromes (Sih et al., 2004).

DISCUSSION

Based on our long-term (15 years) studies of male mating preferences across populations in the Amazon–sailfin–Atlantic molly mating complex, we find considerable geographic variation in male mate recognition and discrimination in mollies. This variation may be associated with the variation among populations in the length of sympatry with the gynogenetic Amazon molly. In some populations, males exhibit consistent species recognition across metrics of assessing mate preference, while in other populations they do not (Table 1; Fig. 2). Males from one population that is recently sympatric to Amazon mollies show more consistency across metrics for measuring mate preferences than males from populations that are sympatric with Amazon mollies, which suggests that male species recognition is a trait that is not fully developed in some sympatric populations. We also find that, whereas there is some amount of variation in male mating preferences across populations, temporal variation on an ecological scale does not appear to be substantial within populations as seen by the relative consistency in the mate choice of populations repeatedly tested for up to 15 years (Table 1; Fig. 2). However, temporal variation on an evolutionary scale among populations in the amount of time in sympathy with Amazons does seem to contribute to variation in male mate choice, as the long-term data from two sympatric populations show that males differ in their consistency and the female traits used for mate preference compared with an introduced recent sympatric population (SP: Table 1; Fig. 2). The patterns of male mate choice in the sympatric populations are consistent within a mating choice metric but there is high variation in male mate choice, and males in these populations need multiple cues to discriminate between conspecific and heterospecific females (Table 1; Fig. 2). In contrast, males from the introduced recent sympatric population are consistent within and between metrics of mate choice. Our data for the introduced sympatric population (Table 1) are interesting because the populations of sailfin molly were first introduced from allopatric populations around 1941 and Amazon mollies were introduced in 1946. Until four years ago (C.R. Gabor, personal observation), Amazons were very rarely found in this population. Now they are as common or more common than sailfin mollies (C.R. Gabor, personal observation). Our experiments were performed before Amazon mollies were common in this population and, while males generally show a conspecific preference, they do not show the additional mating barrier of a preference for small females found in males from one of the sympatric populations (AB: Table 2). Furthermore, males from the
introduced sympatric population do not seem to use multiple cues for mate choice whereas those from sympatry do. These results suggest that multiple pre-mating barriers have arisen in populations that have been in sympatry for longer.

By examining a broader pattern of mating preferences across populations, we gain an understanding of what barriers to heterospecific matings appear and how they may change over time. Specifically, there may be an effect of population-specific selection regimes on mate choice because males in sympatry generally face a conflict between species and mate quality recognition cues (Pfenng, 1998; Gumm and Gabor, 2005). Male mollies usually prefer larger females (Gabor, 1999), as larger females are more fecund except when size cues are also species recognition cues (Gumm and Gabor, 2005). In the case of Amazon mollies, Amazons on average are larger than sailfin molly females and show more variation in size (Gumm and Gabor, 2005; Gabor et al., 2010). Gabor et al. (2010) explored male mate preference for larger size conspecific females using preference functions with model females. They found that males from one sympatric population (VG: Table 2) preferred larger to smaller females but males from a second sympatric population (AB: Table 2) preferred smaller to larger conspecific females. This second population also showed a conspecific mating preference in a simultaneous mate choice design when Amazons were larger, whereas males from no other population maintained their conspecific preference under these conditions (Gumm and Gabor, 2005) (Table 2).

In contrast, male sailfin mollies from an allopatric population (LA: Table 2) preferred to mate with larger Amazon mollies than smaller conspecifics. Males from this same population also preferred to mate with larger conspecifics than smaller ones (Gabor, 1999). Moreover, the sympatric population (AB) that prefers smaller females also has the additional reproductive barrier that may take advantage of an increase in release in KT during mating to aid in species recognition (Table 1), whereas two allopatric populations (C.R. Gabor et al., unpublished data) and the sympatric population of Atlantic mollies (RT: Table 1) do not. The size preference and KT response may provide additional reproductive barriers that have yet to evolve in the other sympatric populations studied to date. It is possible that the preference exhibited by males is changing, on an evolutionary scale, while in sympathy,

<table>
<thead>
<tr>
<th>Publication</th>
<th>Allopatry (LA)</th>
<th>Introduced/recent sympatry (SP)</th>
<th>Sympatry 1 (AB)</th>
<th>Sympatry 2 (VG)</th>
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*Note*: Regular font summarizes results for mate-choice studies (gonopodial thrusts) whereas italic font summarizes results for association preference studies. Figure 1 shows the locations of the populations.
and that only one of the two sympatric populations tested (AB) has diverged to show these changes. In sum, we find that male sailfin mollies show species recognition and discrimination when they have access to multiple cues (i.e. visual, chemical, and tactile) of the females. Moreover, our results suggest that size and KT response are later-evolving species recognition cues. If this is the case, then across time (multiple generations) we could predict to see this change in other sympatric populations, given the presence of enough genetic variation in male preferences.

**Maintenance of Amazon mollies**

One central question that can be explored with our long-term data is: What mechanisms are responsible for explaining the paradoxical maintenance of this mating complex over hundreds of thousands of generations? Theoretically, given the two-fold reproductive advantage of the asexual Amazon mollies over the bisexual parent species, as well as the lack of direct fitness benefits for males mating with Amazon mollies, we expect that this gynogenetic mating complex could not be favoured by selection. Our data suggest that reproductive character displacement (Gabor and Ryan, 2001) of male mate choice may be the first step to prevent the sexual parasite from taking over a population but that natural selection may be important in the maintenance of the co-existence of these species. For example, a few studies have found that sailfin mollies are better at foraging in the short term than Amazon mollies (Alberici da Barbiano *et al.*, 2010; Fisher and Schlupp, 2010; Tobler and Schlupp, 2010), and intraspecific competition in sailfin mollies is stronger than interspecific competition between sailfin and Amazon mollies in juveniles (L. Alberici da Barbiano *et al.*, submitted). By combining the results of mate choice studies and foraging studies, we can infer that the combination of natural and sexual selection allows for the maintenance of this mating complex.

**Conclusions and future work**

As we have summarized in the present article, the amount of time that a selective pressure is present (i.e. sympatry with a closely related species or sexual parasite) is an important aspect to consider when evaluating the evolution of male mate choice. In the case of the discussed gynogenetic mating complex, the time that males have been in sympathy with the gynogenetic females could be confirmed by using molecular techniques. In addition to the aforementioned mechanisms, co-existence between the sexual and asexual species might be maintained via metapopulation dynamics (Kokko *et al.*, 2008). If that were the case, we could imagine a scenario where sympatric populations go locally extinct stochastically and others are colonized. Over a short period of time, ‘new’ sympatric populations are formed due to colonization of Amazons, and some ‘old’ sympatric populations are maintained. It is possible that the variation in behaviour recorded among sympatric populations is a direct consequence of the amount of time spent in sympathy. Some populations like the sympatric AB population might have, by chance, persisted longer than others, like the sympatric VG population (Tables 1 and 2; Fig. 2). In this scenario, males from some populations have had enough time (and perhaps enough standing genetic variation) to allow for the evolution of particular mechanisms that facilitate conspecific preference, whereas the evolution for conspecific preference in other populations was interrupted by stochastic events and later resumed. The hypothesis that time since sympathy could explain the recorded mate preference in males of this complex can be addressed by considering population genealogies.
and using Approximate Bayesian Computation models to estimate the age of the populations (time from divergence between populations) and patterns of historical migration among populations.

The results of this study indicate that by examining mate choice using different metrics in the same populations over time, it has been possible to examine both the change in reproductive isolation barriers and determine the consistency of mating preferences. Few, if any, prior studies re-examine the same mating preferences in populations across time and space. Such an approach allows us to consider the importance of changes in (or consistency in) the expression of mate choice traits, which can lead to a better understanding of the mechanisms of reproductive isolation. Finally, these results indicate that only considering mate choice from one or a few populations is not likely to provide insights into the mating preference of the whole species.

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