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Converging or diverging? Shape coevolution between a sperm-dependent asexual and its sexual hosts

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Asexual species, despite lacking recombination, can evolve in response to environmental changes and influence the evolutionary trajectory of coexisting sexual species. Gynogenesis, where asexual females rely on sperm from males of a different species, offers a unique perspective on the eco-evolutionary dynamics between asexual females and their sexual hosts. The Amazon molly, *Poecilia formosa*, is a gynogenetic species that primarily uses sperm from two sympatric sexual species: the sailfin molly (*P. latipinna*) and the Atlantic molly (*P. mexicana*). To understand shape variation in an asexual species relative to their sexual hosts, we analysed shape variation among wild Amazon mollies and their sexual hosts. We tested three hypotheses: (i) Amazon mollies mimic their sexual hosts to enhance mating opportunities (sexual mimicry hypothesis); (ii) ecological interactions or male mate choice drive morphological divergence (character displacement hypothesis); and (iii) Amazon mollies exhibit random shape variation due to their asexual nature (null hypothesis). Our findings revealed significant shape variation in Amazon mollies, which differ from their sexual hosts in a host-specific manner (e.g. Amazon mollies with *P. latipinna* resemble *P. mexicana* and vice versa), supporting character displacement at the interspecific level in a sexual–asexual system.

1. Introduction

Interspecies interactions can affect organisms' evolutionary trajectories [1,2]. The dynamics of interspecies interactions are particularly relevant in species that rely on other species to reproduce (i.e. sexual parasitism) [3,4]. One form of sexual parasitism, present in approximately 50 asexual vertebrates [5], is sperm-dependent parthenogenesis (gynogenesis). Gynogenetic species are female-only species and lack recombination, producing unreduced eggs that need to be activated by the sperm of a related species [6]. Sperm interacts with the egg to trigger embryogenesis, but the male pronucleus seems to fail to decondense after fertilization, resulting in paternal genome elimination [7,8] and embryo development based on only female genetic information,

producing daughters genetically identical to their mothers [6]. Gynogenetic species are often of hybrid origin and typically coexist with either one or both of their parental ancestors, which they rely upon for sperm [3,6].

Multiple constraints limit the evolution of gynogenetic species. The phenotypic expression of asexual hybrids is influenced by the combination of genomes from the parental species [3], and phenotypic evolution is constrained by the lack of recombination. Sperm dependency also creates a unique ecological relationship, where gynogenetic females must retain access to sperm without outcompeting the host species [9]. Finally, the need for interactions with interspecific males also creates an evolutionary constraint because gynogenetic species cannot diverge to the extent that sperm donor males might no longer recognize them as potential mates, thereby losing access to sperm. Furthermore, gynogenetic females are likely competing with each other and the sexual females for access to males and would benefit from being recognizable and attractive to males of the host species [3,10]. Thus, gynogenetic systems are generally fruitful models for understanding how male mate choice, frequency dependency, social structure, interspecies interactions and evolutionary history can influence sexual selection [3,11].

Theory predicts that moderate male mate discrimination and preference for sexual species are necessary to maintain demographic stability in gynogenetic systems [12,13]. This prediction is confirmed by empirical evidence showing a strong preference for conspecific females over asexual ones in several fish sexual–asexual systems (reviewed in Schlupp [14]). In addition, several studies in gynogenetic systems show that sperm access can be a limiting factor for asexual females, with males showing higher sperm allocation when mating with a conspecific female than to a gynogenetic female [15–22]. In a sperm-limited gynogenetic system, asexual females may increase their mating opportunities and reproductive success by evolving to look as similar as possible to their sexual hosts, a mechanism known as interspecific sexual mimicry. This occurs when organisms mimic sexual traits (e.g. morphology, coloration, displays, mating strategies) of a different species to increase their reproductive success [23]. Sexual mimicry has been shown for one sperm-dependent system, where male *Poeciliopsis lucida* choose between conspecific females and hybridogenetic (i.e. sperm-dependent but with renewal of one genomic set, usually paternal, every generation, also known as hemiclonality [3]) *Poeciliopsis monacha-lucida* females. *P. monacha-lucida* hybridogenetic females that resembled *P. lucida* females—via sexual mimicry of genital coloration and patterning—have greater mating success [23]. Nonetheless, sexual *P. lucida* males strongly prefer conspecific females [17]. Another example of sexual mimicry occurs in the light-coloured pied flycatchers (*Ficedula hypoleuca*), which resemble the plumage coloration of collared flycatchers (*F. albicollis*) females. Light-coloured males, which are female-like, are treated as females by territorial collared flycatcher males and can establish territories closer to collared flycatchers' territories when compared to dark-coloured non-mimicking males [24,25].

While the sexual mimicry hypothesis predicts asexual females resembling local host females, other social and ecological interactions may exert selection on asexual females to diverge from local host females, a phenomenon known as character displacement. During character displacement, sympatric species diverge in relevant phenotypic traits to reduce costly interspecific interactions, facilitating coexistence [26]. In the context of gynogenetic systems, selection for character displacement may arise through ecological competition between asexuals and sexuals (e.g. for food or habitat [27]), through agonistic interactions between asexual and sexual females [28] or through intersexual selection [29]. In this case, intersexual selection may drive character displacement if males exhibit sexual preferences for novel female traits, whereas asexual females that are distinct from local host females are preferred by males [30]. Although the specific mechanisms are often unclear, there is both theoretical [12,31] and empirical [32] evidence for divergence between sympatric sexual and asexual forms.

The Amazon molly, *Poecilia formosa*, is a gynogenetic species distributed in coastal waters from southern Texas, USA, to northeastern Mexico (electronic supplementary material 01, figure S1) [33]. Amazon mollies originated via a single hybridization event between a male sailfin molly (*P. latipinna*) and a female Atlantic molly (*P. mexicana*) that happened between 100 and 120 thousand years ago near present-day Tampico, Mexico [34–37]. Although they can use sperm from multiple sexual hosts in nature [38], Amazon mollies are mostly syntopic (i.e. coexist spatially and temporally) with one (or both) of their parental species, being ecologically very similar [39–41]. In the Amazon molly system, there is strong evidence that both *P. latipinna* and *P. mexicana* males can distinguish between conspecific sexual females and heterospecific Amazon mollies [14,42–44] and often show a preference for conspecific females, particularly during peak reproductive season [45]. This conspecific preference is also reflected in the higher rates of pregnancy in sexual females [43] and higher sperm allocation from males to conspecific females when compared to asexual Amazon mollies [15,18,19,21], suggesting that Amazon mollies may be sperm-limited in the wild.

Given the general male preference for conspecifics and sperm limitation, Amazon molly females should benefit from any mechanism that increases their access to sperm, particularly by weakening or circumventing male mate choice. For instance, Amazon mollies are more aggressive towards *P. latipinna* females than vice versa [46,47]. Amazon mollies also show kin recognition among clones based on body shape differences, increasing their aggression towards non-kin individuals [48]. Sexual mimicry could be another mechanism by which Amazon mollies could increase their reproductive success by looking similar to local sexual females in traits that influence male mate choice. Under the 'sexual mimicry hypothesis' (figure 1a), we predict that selection would favour clonal lineages that express body shape phenotypes that resemble their local heterospecific females, resulting in Amazon mollies looking as similar as possible to the females of the sexual host species with which they coexist locally.

Alternatively, ecological divergence (i.e. habitat specialization, diet differences) and/or interactions among sexual and asexual females (e.g. kin recognition, differential aggressiveness) may push Amazon mollies and their local sexual hosts to opposite areas of the phenotypic space, representing an opposite evolutionary force to potential sexual mimicry. Strong favourable responses to novel traits have been widely documented in poeciliids [49,50]. Furthermore, pre-existing biases are widespread in the family [51,52], including in males [53,54]. All those mechanisms can underlie the 'character displacement hypothesis' (figure 1b), which predicts that selection has acted to maintain Amazon mollies that are dissimilar from its local sexual host females. Finally, given its asexual nature and relatively recent origin [37], Amazon mollies may have diverged very little or randomly, regardless of the local sexual host with whom they coexist ('null hypothesis'; figure 1c). Here, we explore
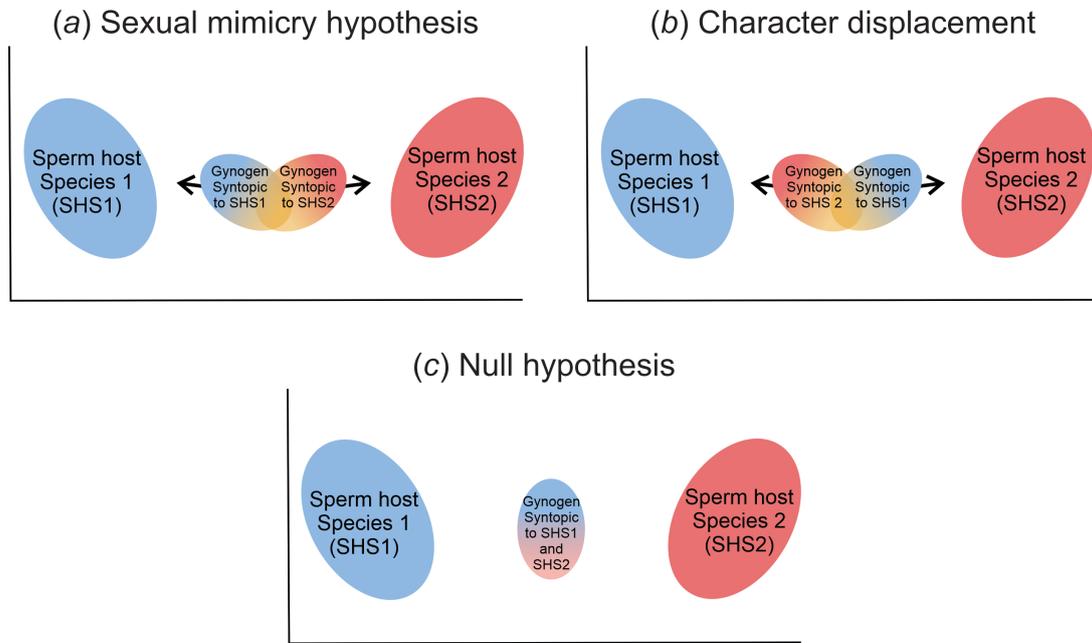
Figure 1. Schematic of predictions under the three different hypotheses tested in this study. (*a*) Under the sexual mimicry hypothesis, gynogenetic females evolved to look as similar as possible to their sexual hosts, given the male preferences for females of their species. (*b*) Under the character displacement hypothesis, gynogenetic females look dissimilar to females of their sexual hosts, given the potential ecological differences between sexual and asexual females. (*c*) Under the null hypothesis, no directed morphological divergence occurred in the asexual females.

these contrasting hypotheses based on body shape variation, a trait that has been shown to affect mate choice in poeciliids [55–57] and Amazon mollies clonal recognition [48]. We tested those contrasting hypotheses by comparing body shape variation among combinations of wild Amazon molly populations sympatric or allopatric with its main sexual hosts across their range.

2. Methods

(a) Sampling

To address these questions, we leveraged two independent and complementary datasets that used slightly different methodological approaches. The first dataset included a balanced sampling of *P. formosa* (*n* = 212) and its host species from six natural populations in Texas, USA, and Tamaulipas, Mexico, and one common-garden-reared population (since 1994) from a site where *P. formosa* coexists with both host species. In this first dataset (dataset I), geometric morphometric analyses were based on external landmarks on lateral photographs of live specimens taken in the field (figure 2*a*). The second dataset (dataset II) included a larger sample size in terms of the number of sites (*n* = 18) and specimens (*n* = 678). Despite a larger sample size, dataset II is often unbalanced in terms of species sampled within collection locations (reflecting the relative frequency of species at the time of collection). For dataset II, specimens were fixed upon collection, and geometric morphometric analyses were based on internal landmarks on lateral radiographs of the preserved specimens (electronic supplementary material, figure S2.2). All females included in each of the two datasets were sexually mature (>30 mm). Both approaches yielded qualitatively remarkably similar results. Given the more balanced distribution of specimens across sample sites in dataset I, we present the details of the methods and findings based on dataset I. The methods and results for dataset II are presented in the electronic supplementary material 02.

(b) Geometric morphometric data processing

For dataset I, sampling was conducted in September 2001 throughout Texas, USA, and Tamaulipas, Mexico, using a 6.4 mm mesh, 7.6 m × 1.2 m standard minnow seine. The fish were anaesthetized using tricaine methanesulfonate (MS-222) and photographed on-site. At some sites, Amazon mollies lived syntopically with *P. mexicana*; at others, they coexisted with *P. latipinna* (electronic supplementary material 01, figure S1, table S1).

We acquired images from 212 individuals (120 *P. formosa* females, 102 from wild populations and 18 from one stock population (IV/5); 58 wild *P. latipinna* females and 34 wild *P. mexicana* females) from seven localities across their natural range and different combinations between Amazon mollies and its sexual hosts (electronic supplementary material, figure S1, table S1). We classified sampling points by the presence of Amazon mollies and their syntopy with its sexual hosts, either *P. latipinna* mainly in Texas, USA, or *P. mexicana*, from Tamaulipas, Mexico. Digital photographs were taken on-site on a laminated millimetre grid using an Olympus Camedia 2500L digital camera. Camera set-up, light condition and individual positioning (left side of the individual positioned at the centre of the laminated grid) were standardized as much as possible to avoid photographic errors across sampling points. To have a shape representation from *P. formosa* females from sites where Amazon mollies coexist with
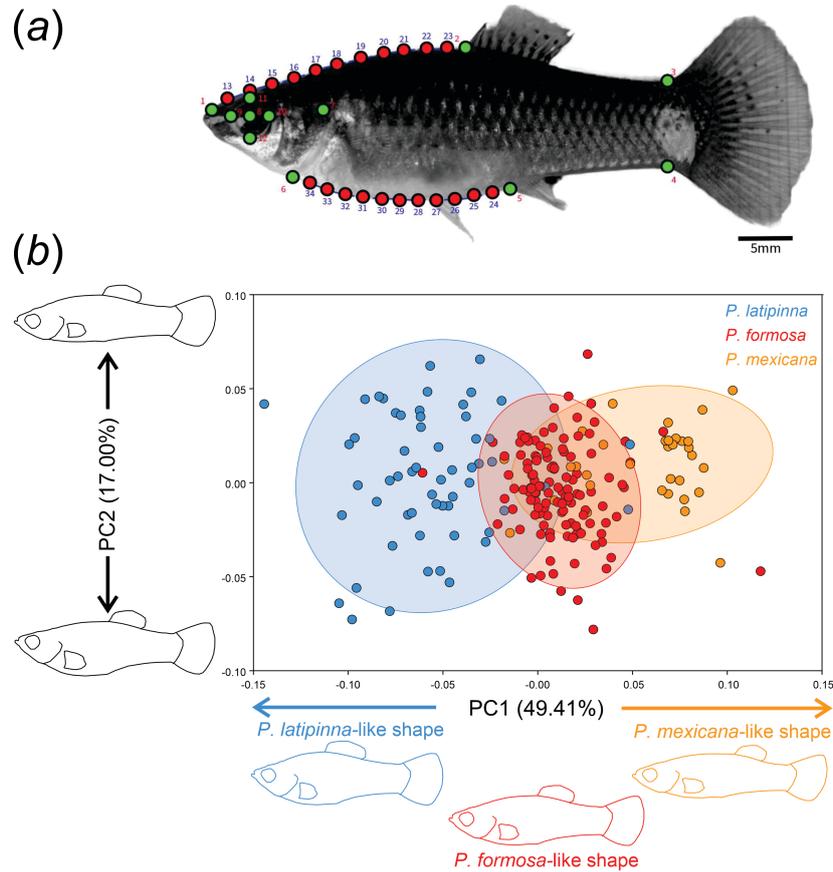
Figure 2. Landmark configuration for shape variation and principal component analysis. (*a*) Location of landmarks (green circles) and semi-landmarks (red circles) used for shape quantification of Amazon mollies (*P. formosa*) and females of its sexual hosts (*P. latipinna* and *P. mexicana*). Landmarks: (1) anterior tip of the snout, (2) anterior insertion of the dorsal fin, (3) dorsal insertion of the caudal fin, (4) ventral insertion of the caudal fin, (5) anterior insertion of the anal fin, (6) ventral margin of the operculum, (7) dorsal margin of the operculum, (8) centre of the eye, (9–12) anterior, posterior, dorsal and ventral margins of the eye. Semi-landmarks: (13–23) semi-landmarks evenly spaced along the dorsal edge of the body from the anterior tip of the snout to the anterior insertion of the dorsal fin, (24–34) semi-landmarks evenly spaced along the ventral edge of the body from the anterior insertion of the anal fin to the ventral margin of the operculum. (*b*) Principal component analysis (PC1 versus PC2) for size-corrected shape configuration in Amazon mollies (*P. formosa*) and females from its sexual hosts (*P. latipinna* and *P. mexicana*). Ellipses with a 90% confidence interval. Shapes at the bottom represent the mean shape configuration along PC1 and PC2. PC1 −0.1 for *P. latipinna*, 0.0 for *P. formosa* and 0.1 for *P. mexicana*. PC2 shapes are shown for −0.05 and 0.05.

both *P. latipinna* and *P. mexicana*, we added one population from a laboratory stock (electronic supplementary material 01, figure S1, table S1). All photos were taken with a Nikon D5200 camera with a Nikon DX 18–55 mm lens but otherwise treated the same way as the field photos.

Twelve landmarks and 22 semi-landmarks (figure 2*a*) were digitized using TPSDig v. 2.30 [58]. The software TPSUtil v. 1.82 [58] was used to compile and convert the image file into a '.tps' file for downstream analysis in MorphoJ v. 1.07a [59]. In MorphoJ, we superimposed the landmark configurations using generalized Procrustes analysis [60], generated a covariance matrix and performed all geometric morphometric analyses. We conducted a multivariate regression of Procrustes coordinates (shape) and the centroid size (size) to account for allometric effects on shape variation [61]. We then used the residuals of this regression as size-corrected shape data for the subsequent analysis using MorphoJ.

(c) Geometric morphometric analysis

All geometric morphometric analysis was performed using MorphoJ v. 1.07a. We first used principal component analysis (PCA) to characterize the overall shape differences between females of *P. latipinna* (sexual host), *P. mexicana* (sexual host) and *P. formosa* (asexual sperm-parasite). For PCA, we selected the minimum number of principal axes that together explained at least 75% of the overall shape variation (electronic supplementary material 01, figure S3).

We investigated whether Amazon mollies have a shape similar (sexual mimicry hypothesis), dissimilar (character displacement hypothesis) or had little/random morphological variation (null hypothesis) in relation to the sexual hosts with whom they coexist. We compared shape differences among these groups using a canonical variate analysis (CVA) to determine the effectiveness of variation in predicting *a priori* group assignments using a T^2 Hotelling test with 1000 permutations with Bonferroni multiple correction. This approach tests against the null hypothesis that group averages have no shape differences. A Procrustes pairwise distance matrix was used as a shape distance matrix among groups. For individual pair-to-pair comparisons, we used discriminant function analysis (DFA). We performed cross-validation and permutation tests (1000 permutations each) for all comparisons between groups to statistically evaluate the discriminant scores, using Procrustes distances. From those permutation tests, an individual classification/misclassification table was generated to assess the percentage of individuals
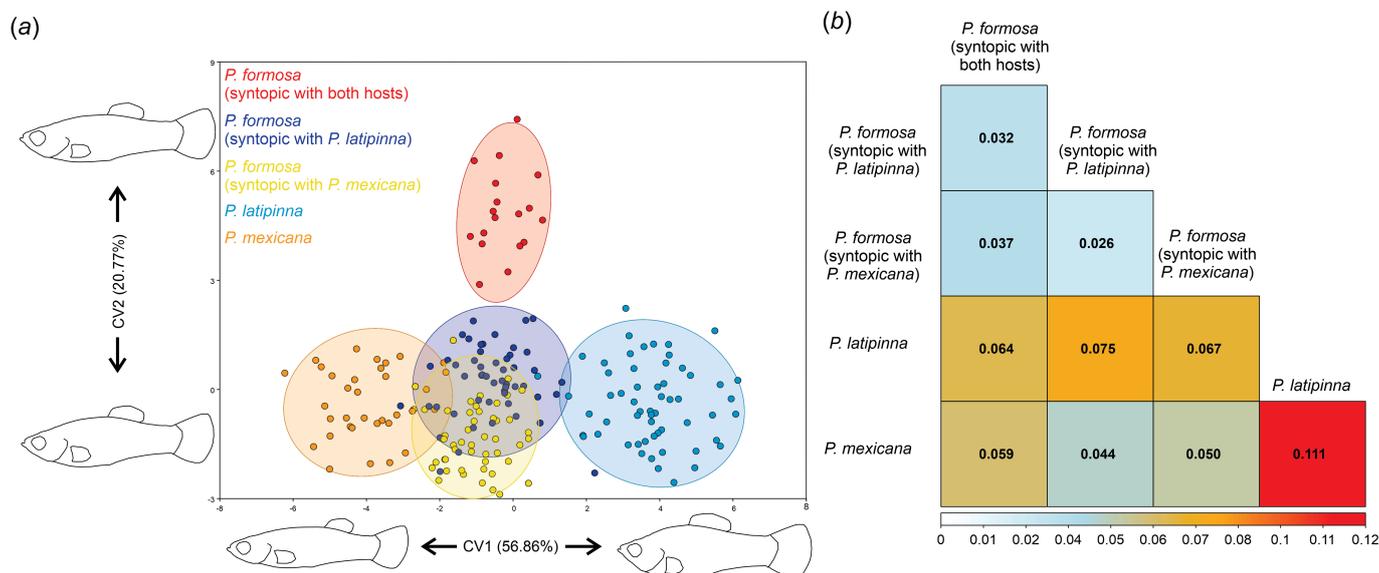
Figure 3. Shape variation and distances among Amazon mollies and their sexual hosts. (*a*) Canonical variate analysis for size-corrected shape configuration in Amazon mollies (*P. formosa*) and females from its sexual hosts (*P. latipinna* and *P. mexicana*). CV1 shape configurations are shown for −5.0 and 5.0. CV2 shapes are shown for −2.0 and 8.0. (*b*) Pairwise, Procrustes distance values in Amazon mollies females from its sperm donors. All pairwise comparisons had *p*-values <0.001. Procrustes distance values between groups are shown in (*b*).

per group correctly assigned by the DFA after 1000 permutations. We reran a CVA as described above but with a dataset containing only Amazon mollies to check for shape differences among Amazon mollies according to their syntopic sexual hosts. A warped-outline drawing graphic was chosen to visualize shape variation.

3. Results

The PCA of overall size-corrected shape differences showed *P. formosa* individuals to be generally intermediate in body shape compared to its parental species, *P. latipinna* and *P. mexicana* (figure 2). This was expected, given the hybrid origin of the Amazon mollies. The first axis (PC1 in figure 2) explained almost half (49.41%) of the total shape variation. *P. latipinna* individuals are grouped in the negative values of PC1, generally showing a deep body, with anterior insertion of a long dorsal fin, deep head profile, small snout length and deep caudal peduncle. *P. mexicana* individuals are grouped in the positive values of PC1, generally showing a more fusiform shape, with posterior insertion of a short dorsal fin, a longer snout and a shallower caudal peduncle. *P. formosa* individuals were generally placed between *P. latipinna* and *P. mexicana*, typically showing intermediate shape traits between *P. latipinna* and *P. mexicana*. PC2 explained 17% of the total shape variation but did not show a clear distinction between groups, and its shape variation was mainly related to the curvature of the ventral side of the fish, which may be related to body condition or the pregnancy status of the females (figure 2). Inspection of PC3 (explaining 12.07% of the overall variation) did not reveal any further differentiation between species or Amazon molly populations (electronic supplementary material 01, figure S2).

Amazon mollies overlap in morpho-space with both parental species, but they tend to have a slightly higher overlap in shape with *P. mexicana* than with *P. latipinna* females. This result was corroborated by our DFA cross-validation tests, which showed that when compared to *P. mexicana*, 92.5% (versus 95% with *P. latipinna*) of the Amazon mollies are correctly assigned to the Amazon molly group. Only 82.35% of the *P. mexicana* (versus 89.66% of *P. latipinna*) females are correctly assigned to their group compared to the Amazon mollies (electronic supplementary material 01, figure S4a).

The CVA comparing females of *P. latipinna*, *P. mexicana* and *P. formosa* (grouped according to the sexual host they are syntopic with) also supported the intermediate shape phenotype of Amazon mollies relative to its parental species (figure 3). All pairwise comparisons were highly significant at both distances using permutation tests, even after Bonferroni correction (*p* < 0.001). CV1 explained 56.86% of the overall variation. It reflected the overall shape differences observed in the PCA between *P. latipinna* and *P. mexicana*, with Amazon mollies showing an intermediate shape between *P. latipinna* and *P. mexicana*. CV2 explained 20.77% of the shape variation and isolated Amazon mollies that coexist with both sexual hosts from the remaining groups, with the former individuals showing a more streamlined shape when compared to other Amazon mollies and its parental species (figure 3a).

The matrix of pairwise Procrustes shape distances showed some interesting patterns (figure 3b). As expected, the lowest shape distance values were between Amazon mollies groups (average 0.031) and the highest between *P. latipinna* and *P. mexicana* females (0.111). All comparisons, even between Amazon molly groups, were significant (*p* < 0.001), demonstrating significant shape differences between the *a priori* defined group averages. Amazon mollies that coexist with both sexual hosts were shown to be approximately equally distant from both sexual hosts in body shape (Procrustes shape distance average = 0.06). However, Amazon mollies that coexist with *P. latipinna* are, in shape, approximately 1.7 times as different from *P. latipinna* (Procrustes shape distance = 0.075) as they are from *P. mexicana* (Procrustes shape distance = 0.044), suggesting that Amazon mollies that
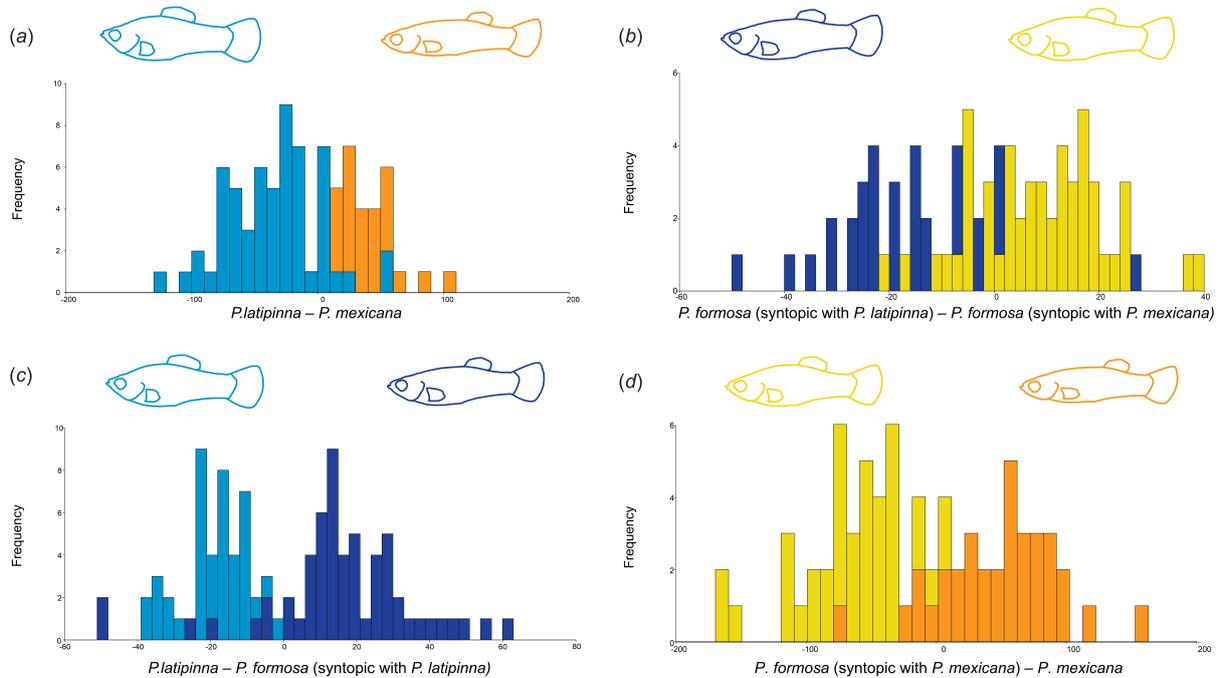
Figure 4. Cross-validation tests (after 1000 permutations) for size-corrected shape differences between Amazon mollies and their sexual hosts. (*a*) Comparison between *P. latipinna* (light blue) and *P. mexicana* (orange). (*b*) Comparison between *P. formosa* females syntopic with *P. latipinna* (dark blue) and *P. formosa* females syntopic with *P. mexicana* (yellow). (*c*) Comparison between *P. latipinna* females (light blue) and *P. formosa* females syntopic with *P. latipinna* (dark blue). (*d*) Comparison between *P. mexicana* (orange) females and *P. formosa* females syntopic with *P. mexicana* (yellow). Shapes at the top of each plot represent average shapes for each group in the comparison. All pairwise comparisons had *p*-values <0.001. Procrustes distance values between groups are shown in figure 3*b*.

are syntopic with *P. latipinna* are in overall shape more similar to *P. mexicana* than *P. latipinna*. Amazon mollies that coexist with *P. mexicana* are also significantly different from *P. mexicana* (Procrustes shape distance = 0.050); however, they are slightly more different from *P. latipinna* females (Procrustes shape distance = 0.067).

Our pairwise DFAs confirmed the results revealed in the PCA and CVA. *P. latipinna* and *P. mexicana* females significantly differ from each other, showing the largest Procrustes shape distance. *P. latipinna* females have deeper bodies, bigger heads, larger caudal peduncle areas, more anterior dorsal fin insertion and larger dorsal fins than *P. mexicana* females (figure 4*a*). Amazon mollies that live with either *P. latipinna* or *P. mexicana* are also significantly different from each other, with the former having a shallower body and smaller head when compared to the latter (figure 4*b*). Following the results in the CVA, Amazon mollies that are syntopic with *P. latipinna* are significantly different from *P. latipinna* females, showing shallower bodies and heads in an overall shape resembling *P. mexicana* females (figure 4*c*). The same is true for Amazon mollies that coexist with *P. mexicana*, which generally show a deeper body shape, resembling the general shape of *P. latipinna* females (figure 4*d*).

Our cross-validation tests corroborated the host-specific scenario in shape differences between Amazon mollies and their sexual hosts. Still, again, the host-specific effect was more robust in Amazon mollies living with *P. latipinna* than with *P. mexicana*. The average number of correctly assigned individuals to their populations was 79.5%. Amazon mollies that are syntopic with *P. latipinna* were correctly classified as *P. formosa* in 90.4% of the cross-validation tests when compared to *P. latipinna* (versus 71.1% when compared to *P. mexicana*), showing a shape resemblance between those Amazon mollies and *P. mexicana*. Amazon mollies syntopic with *P. mexicana* were correctly classified as *P. formosa* in 84% of the tests (versus 92% compared to *P. latipinna*). Interestingly, the Amazon mollies that are syntopic with both sexual hosts had a higher percentage of correctly assigned individuals in comparison to *P. mexicana* (88.9%) than to *P. latipinna* (83.3%). When comparing between Amazon molly groups only, the Amazon mollies that are syntopic to both sexual hosts had a higher percentage of correctly assigned individuals (94.4%) when compared to Amazon mollies syntopic with *P. latipinna* (which generally have a shape more similar to *P. mexicana*) than to the ones syntopic with *P. mexicana* (66.7%) (electronic supplementary material 01, figure S4b). Altogether, those results suggest that contrary to the general pattern of shape resemblance to *P. mexicana*, Amazon mollies that are syntopic with both sexual hosts have higher similarity in shape to *P. latipinna* than to *P. mexicana*. It should be noted that this Amazon molly population, which is syntopic with both host species, is also the only laboratory population.

The CVA results using only Amazon molly individuals further support the existence of shape differences between Amazon molly populations associated with the sexual host (electronic supplementary material 01, figure S5b). CV1 separates Amazon mollies that live with either one or both hosts. Amazon mollies from the former were generally more deep-bodied than the latter. CV2 showed an interesting pattern, where Amazon mollies syntopic with both hosts showed an intermediate shape to Amazon mollies syntopic with either one or the other sexual host. As observed in previous analyses, Amazon mollies syntopic with *P. latipinna* have a more fusiform-like shape, with shallower body depth and head areas, resembling *P. mexicana* individuals. On the other hand, Amazon mollies syntopic with *P. mexicana* are more deep-bodied, with larger heads and deeper peduncle areas, generally resembling *P. latipinna* individuals (electronic supplementary material 01, figure S5). All pairwise comparisons between groups were significant (*p* < 0.001).

Despite the differences in landmark configuration, populations, and sample sizes and composition between datasets, the results for dataset II were remarkably similar to those shown here, with Amazon mollies generally showing an intermediate phenotype between *P. mexicana* and *P. latipinna*. Amazon mollies also showed host-specific shapes across their distribution as observed in dataset I. A full description of the results on dataset II can be found in the electronic supplementary material 02.

4. Discussion

Sperm-dependent asexuality creates a unique eco-evolutionary dynamic between sperm parasites and their sexual hosts. We tested whether the sperm-dependent Amazon molly shows shape variation across their distribution and whether the variation is related to the identity of the local sexual host. Our study revealed that despite their clonal nature and relatively recent origin (circa 100 000 years or 400 000 generations [37]), Amazon mollies significantly differ in shape across populations. Strikingly, Amazon mollies display specific body shapes associated with their local sexual host, with individuals that coexist with either *P. latipinna* or *P. mexicana* being significantly more dissimilar from their local host species than expected.

Several lines of evidence indicate that Amazon mollies may be sperm-limited in the wild [15,18,19,21,43]. Our sexual mimicry hypothesis postulated that Amazon mollies may have evolved to look similar to their local hosts, therefore having an advantage in mating opportunities and reproductive success by thwarting male conspecific preferences for female shape. Although sexual mimicry has been found in other organisms [24], including in poeciliid fishes [23], our data have shown that Amazon mollies are dissimilar, not similar, to their local sexual hosts, locally resembling the other paternal species with whom they coexist, suggesting that sexual mimicry is not a mechanism explaining the body shape evolution of Amazon mollies in the wild.

As our study is based on wild populations, phenotypic plasticity cannot be entirely ruled out. However, the convergence of shape-specific changes in multiple populations likely to differ in environmental conditions and the similar results found between two independent datasets suggests that the shape differences documented here are the product of adaptive evolution. Competition for resources can cause character displacement, pushing sympatric species to different positions of morpho-space compared to their allopatric counterparts [26]. Such ecological character displacement has been documented in a wide variety of taxa and ecological contexts [27,62–65]. In the Amazon molly system, all evidence points towards a high degree of ecological overlap between the asexuals and their sexual hosts. For example, asexuals and sexuals overlap in diet [39], food-finding behaviour [40] and parasite communities [41]. This high degree of ecological overlap is not surprising given the phylogenetic origin of Amazon mollies and their sperm dependency that requires them to be truly syntopic with their sexual hosts. Hence, the tight ecological overlap between the Amazon molly and its hosts sets the stage for intense competition that could drive ecological character displacement in body shape. However, it is important to note that it remains unclear how the divergence in body shape documented here would ultimately reduce competition between Amazon mollies and their sympatric sexual hosts. Further studies need to test how body shape affects the fitness of asexuals in the context of different host species and whether there are yet undocumented microecological differences in the dietary, spatial or temporal niches of coexisting molly species.

Another mechanism that could lead to character displacement is agonistic interactions between sexual and asexual females [28], whereby divergence in female phenotypes may decrease aggressive interactions between species. Makowicz & Schlupp [47] found that *P. latipinna* females and Amazon mollies are equally aggressive to either conspecific or heterospecific females. Amazon mollies, however, sustained their long-term aggression levels, particularly to heterospecific females. Overall, these findings suggest intense female competition between Amazon mollies and their local sexual host females. Yet, the fact that Amazon mollies, not the sexual hosts, are not only the more aggressive species but also the ones facing a cost from being aggressive does not support the notion of selection for phenotypic divergence in the Amazon mollies caused by female competition. If Amazons are more aggressive, sexual females, not Amazon mollies, would be the ones benefiting from phenotypic divergence. The extent of shape divergence between sexual females that coexist or not with Amazon mollies remains to be investigated.

Schlupp *et al.* [66] and Ryan *et al.* [44] argued that given the general tendency for conspecific preference (as reviewed in Schlupp [14] and Gabor *et al.* [67]) an arms race between the males and Amazon mollies may be in place. To circumvent conspecific male mate preference, asexual females may have developed tactics to increase mating opportunities (e.g. female size and receptivity, frequency-dependent effects, and behavioural and morphological changes). This prediction is supported by theoretical models showing that asexual females may have evolved counter-adaptations to male mate discrimination, rendering it costly and making the evolution of strong male mate discrimination unlikely [12]. The divergent host-specific body shape morphologies found here could also be driven by sexual selection acting on body shape variation via male mate choice. We propose the 'sexual novelty hypothesis', where asexual females may have had increased attractiveness to sexual males by being different, tapping into a preference for novelty, commonly found in poeciliids [14,30,49,50,68–76]. Given the genetic constraints associated with the hybrid origin of Amazon mollies, sexual selection via male mate choice could have favoured divergent phenotypes by selecting clones that resemble females of the locally opposite species. Our results indirectly support that prediction, showing host-specific divergent body shape phenotypes across replicates of wild populations, suggesting a standard selective pressure on body shape according to the identity of the local sexual host. Another interesting finding is that the only population included in our sampling that coexists with both sexual hosts (from Tampico, Mexico) is significantly different from the other Amazon molly populations, showing an intermediate body shape between Amazon mollies resembling *P. mexicana* (syntopic with *P. latipinna*) and *P. latipinna* (syntopic to *P. mexicana*) (electronic supplementary material02, figures S2 and S5). This intermediate phenotype in a population with both sexual hosts supports the idea that different host-specific selective pressures act on Amazon molly populations. However, this finding must be interpreted with caution as the population

from sympatry with both *P. mexicana* and *P. latipinna* has been maintained in captivity since 1994 and may have fixed body shape phenotypes that are not necessarily related to its interactions with sexual hosts in the wild.

Although our results indirectly suggest that male mate choice may have been selected for body shape differentiation in Amazon mollies, we did not directly test for male mate preferences between wild allopatric and sympatric Amazon mollies. Makowicz *et al*. [11] found no preference to associate with allopatric or sympatric Amazon mollies in Sailfin and Atlantic mollies. In addition, [77] did not find any preference by sailfin molly males for artificially modified (e.g. dorsal fin, spot pattern and body shape modifications) Amazon molly models based on visual cues. However, none of those findings can rule out the possibility that, on an evolutionary timescale in the wild, male mate choice dynamics in this system have favoured Amazon molly clones that differentiate them from their local sexual hosts. An early male preference for local divergent phenotypes may have favoured Amazon molly clones with a body shape biased toward the opposite parental species, resulting in an increased frequency of 'opposite-like' females across multiple populations. Our results call for further studies integrating mate choice experiments using wild individuals and genomic data to reveal the processes and mechanisms driving the host-specific body shape differences between Amazon molly populations and the origin and maintenance of this sexual–asexual system.

The body shape differences found here were mainly in body depth and insertion of the dorsal fin. Overall, Amazon mollies syntopic with *P. latipinna* are more streamlined while Amazon mollies syntopic with *P. mexicana* are more deep-bodied. Body depth changes in fishes have been previously associated with different predation regimes [56] and differences in water flow [78,79]. In livebearing females, differences in body depth can also be explained by different pregnancy statuses [80]. In our dataset, however, potential pregnancy effects (on PC2 of our PCA) did not show a clear differentiation between species, suggesting that the distribution of (non) pregnant females may have been similar across species in our dataset (the same for dataset II, electronic supplementary material, S2). Instead of biological artefacts, the variation in body depth and insertion of the dorsal fin in the females may reflect traits used during male mate choice. Body depth and dorsal fin length are indeed among the most marked morphological differences between *P. latipinna* (member of the 'sailfin mollies' clade) and *P. mexicana* (member of the 'shortfin mollies' clade), particularly in males [35,81–83]. In addition, differences in body depth have been used as a trait for female mate choice decisions in the livebearing mosquitofish *Gambusia* [56] and *P. mexicana* [55], supporting the idea that minute size-independent differences in body shape can be used as a sexually selected trait in poeciliids [48,55].

Given the lack of meiotic recombination, evolution in asexual species is predicted to be slower when compared to sexual organisms. Effective recombination allows for decreased accumulation of deleterious mutations and quicker combination of beneficial genotypes within a single individual [84]. Sexual reproduction also increases fitness variance at the population level by creating multiple genotypic combinations for selection to act upon [85]. Previous studies have occasionally considered Amazon mollies to be 'frozen F1s', with little or no evolutionary change since the single original hybrid speciation event from which they originated [86,87]. However, our findings reject this and are in line with several studies that have documented behavioural, morphological and genetic/genomic differences among Amazon molly populations, particularly between populations from Tamaulipas, Mexico (its southernmost distribution is usually sympatric with *P. mexicana*), and Texas, USA (its northernmost distribution, sympatric with *P. latipinna*) [48,88,89]. Given the single origin of the Amazon molly, variability across populations can be generated via *de novo* mutation, gene conversion [90] and differential levels of paternal introgression [37]. Parental introgression can occur either via microchromosomes [35] or whole genomes [91], which occasionally lead to triploid clones. Regardless of the mechanism, the host-specific morphologies found here add to the fact that Amazon molly populations, despite their functionally ameiotic reproduction [92] and their overall closer resemblance to *P. mexicana* (its maternal ancestor), are highly variable, with a marked divergence between northern and southern populations.

In conclusion, our study agrees with the increasing body of evidence challenging the paradigm of limited evolutionary potential in asexual organisms [93,94], with either fast genetic-based evolution and/or plasticity aiding long-term survival and increased fitness in the wild [95,96]. Our study suggests a more intimate and potentially often overlooked evolutionary dynamic between sperm-dependent organisms and their sexual hosts. It provides a remarkable example of how interspecific interactions can drive short-term evolutionary change, even in asexual species.

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