

RESEARCH ARTICLE

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Mating system drives negative associations between morphological features in *Schistosomatidae*

Sophie Beltran^{1*}, Yves Desdevises², Julien Portela¹, Jérôme Boissier^{1*}

Abstract

Background: Sexual morphological features are known to be associated with the mating systems of several animal groups. However, it has been suggested that morphological features other than sexual characteristics could also be constrained by the mating system as a consequence of negative associations. *Schistosomatidae* are parasitic organisms that vary in mating system and can thus be used to explore links between the mating system and negative associations with morphological features.

Results: A comparative analysis of *Schistosomatidae* morphological features revealed an association between the mating system (monogamous versus polygynandrous) and morphological characteristics of reproduction, nutrition, and locomotion.

Conclusions: The mating system drives negative associations between somatic and sexual morphological features. In monogamous species, males display a lower investment in sexual tissues and a higher commitment of resources to tissues involved in female transport, protection, and feeding assistance. In contrast, males of polygynandrous species invest to a greater extent in sexual tissues at the cost of reduced commitment to female care.

Background

A mating system reflects the manner in which members of an animal society are structured with respect to sexual behaviour. Three mating systems are generally recognised: monogamy, polygamy, and polygynandry (or promiscuity). In monogamous species, males and females have only one sexual partner at any given time. In polygamous species, one male has a mating relationship with several females (*i.e.*, polygyny) or one female has a mating relationship with several males (*i.e.*, polyandry). Finally, polygynandry is a mating system in which any male mates with any female. Specific morphological features are known to be associated with the mating systems of several animal groups, including primates [1,2], bats [3], birds [4-6], rodents [7], teleost fishes [8], amphibians [9], and insects [10,11]. Logically, as a consequence of sexual selection, such

morphological features mainly involve primary or secondary sexual characteristics. However, it has been suggested that morphological features other than such characteristics could also be constrained by the mating system, reflecting evolutionary trade-offs between effective mating and bodily phenotype [3]. Previous authors indicated that males of bat species with mating systems based on female promiscuity had smaller brains and larger testes, whereas species with mating systems involving female fidelity were endowed with larger brains and smaller testes. This pattern was interpreted as an investment trade-off between two metabolically expensive organs [3]. Such an “expensive sexual tissue” hypothesis proposes that more intense sexual selection will affect the evolution of energy-demanding tissue and associated functions as a result of negative association with costly sexual organs, ornaments or armaments [3]. Although this hypothesis has been proven in bats [3], no such link has been demonstrated in mammals [12].

Schistosomes (Trematoda: Schistosomatidae) are endoparasites of birds and mammals [13]. The ~100 species of schistosomes are unusual among the ~18,000

* Correspondence: beltran.sophie@hotmail.fr; boissier@univ-perp.fr

¹Laboratoire de Parasitologie Fonctionnelle et Evolutive, Centre de Biologie et d'Écologie Tropicale et Méditerranéenne, UMR 5244 CNRS-UPVD, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France
Full list of author information is available at the end of the article

species of the subclass Digenea because, unlike other digeneans (which are usually hermaphroditic), schistosomes are of two separate sexes. More importantly, schistosomes are the only parasitic organisms that show variability in mating systems. Three such systems have been identified in these worms [14]: (1) Monogamy occurs in ~30 species and, in these species, worm pairs consisting of only one female and only one male can be observed either *in vivo* or after experimental recovery. Moreover, the monogamous female needs the continuous presence of a male to maintain sexual activity, making monogamy compulsory. However, monogamy does not imply faithfulness. Mate changes can occur, as have been shown in the genus *Schistosoma* [15,16]; this means that schistosomes are socially but not genetically monogamous [17]. (ii) Polygyny occurs in ~4 species and, in these species, one male monopolizes more than one female, with other males having no access to these females. (iii) Polygynandry occurs in ~66 species; males and females are never seen *in copula in vivo* (i.e., males and females mate with several partners of the opposite sex over a given period of time). In contrast to monogamous female schistosomes, polygynandrous females are able to attain sexual maturity and to lay eggs even if a male is not continuously present [18]. Schistosomes are therefore the only parasitic organisms that can be used to explore possible links between a chosen mating system and a negative association with a morphological feature. The goals of the present work are (i) to determine if, as a consequence of mate competition, male polygynandrous schistosomes invest more energy (as measured by testis size) in their reproductive organs than do monogamous males; and (ii) to establish whether any negative association between investment in

sexual and somatic tissues can be identified. Our prediction was that the larger the investment in sexual tissue, the smaller would be the investment in locomotor and nutritive functions, as measured by relative sucker size and oesophagus length, respectively.

Methods

Data collection

A total of 28 species were included in this study, a number that is limited by DNA sequence information required for the phylogenetic reconstruction used in the comparative analyses. Nineteen species from six genera are monogamous, and nine species from six genera are polygynandrous. DNA sequence information is available for only two polygynous species, which were therefore not included in the analysis. Data on morphological features were collected from published parasite descriptions; these measurements are summarized in Additional file 1, Table S1. The surface area of each organ was calculated from the length (*l*) and the width (*w*) of the organ using the ellipsis surface area formula ($l \times w \times \pi/4$). The relative organ length is the length of the organ divided by the body length, and the relative organ surface area is the surface area of the organ divided by the body surface area. We identified three groups of morphological features according to their functions (Figure 1):

1. The “reproduction group” constitutes sexual morphological features of female and male schistosomes. For females, we recorded the relative seminal receptacle surface area (seminal receptacle surface area divided by the total surface area of the body) and the relative ovary length (ovary length divided by the overall length of the body). For males, we determined the number of testes,

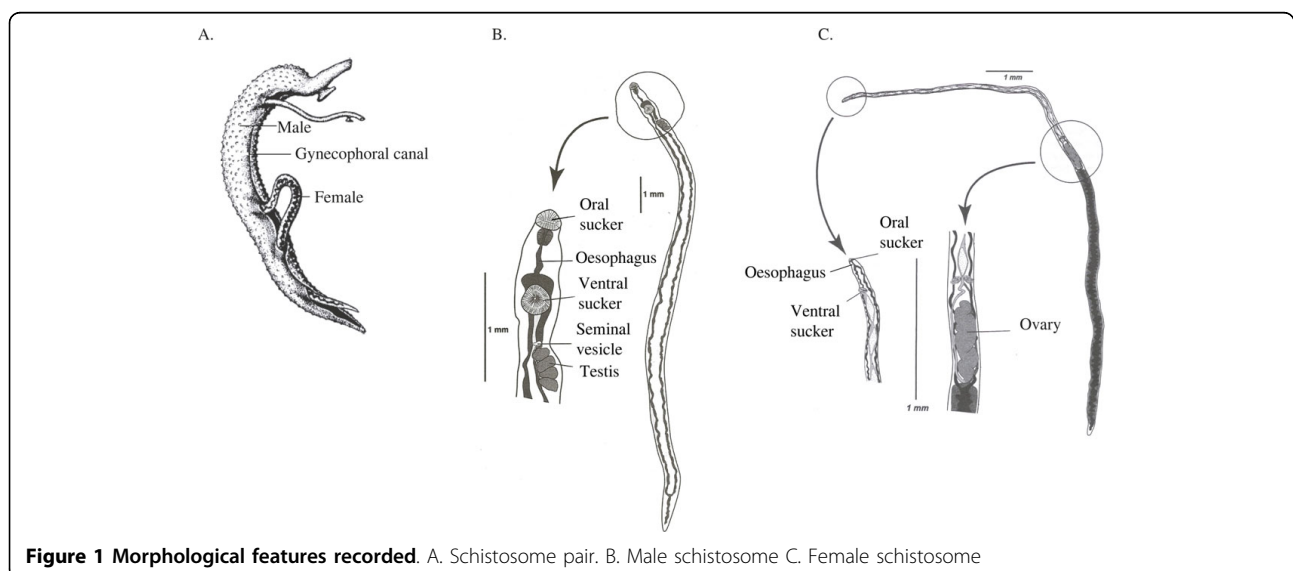


Figure 1 Morphological features recorded. A. Schistosome pair. B. Male schistosome. C. Female schistosome

and measured the relative seminal vesicle surface area (seminal vesicle surface area divided by the total surface area of the body) and relative testes surface area (total testes surface area divided by body surface area). We also recorded the relative male gynecophoral canal length (length of the gynecophoral canal divided by the overall length of the body). The gynecophoral canal is a groove on the ventral surface of the male in which the female is held during copulation.

2. The “nutrition group” constitutes somatic morphological features of female and male schistosomes involved in nutrition. For males and females, we recorded the relative oesophagus length (oesophagus length divided by the overall length of the body), which has implications for the transport of food toward gut caecae.

3. The “locomotion group” contains somatic morphological features of female and male schistosomes involved in locomotion. Schistosomes, like all digeneans, possess an oral sucker and a ventral sucker, or acetabulum. Locomotion is achieved by alternate attachment of the suckers on internal host surfaces [19]. For males and females, we measured relative oral and ventral sucker surface areas (sucker surface area divided by the total surface area of the body). We also computed male/female relative sucker-surface-area ratios.

Note that, in addition to its inclusion in the reproduction group, the male gynecophoral canal could appear in all three morphological groups because of its potential involvement in female nutrition (through transtegumental transfer of substances) [20], female sexual maturation [21], female locomotion [22] and possibly mate guarding and female protection against the host immune system [14,23].

Comparative analyses

To control for phylogeny, we performed a phylogenetic reconstruction among the *Schistosomatidae* species using published DNA sequences of complete 18S and 28S rDNA genes, and a partial sequence of the cytochrome oxidase 1 (CO1) mtDNA gene (see Additional file 2, Table S1). Sequences were aligned using MAFFT, version 5 [24,25], and were improved by eye using Se-Align v2.0a11 [26]. After deleting ambiguous regions from the alignments, the final lengths of DNA sequences were 1653 bp (18S), 3741 bp (28S) and 1095 bp (CO1). Because not all species investigated were sequenced for all genes used, we constructed trees from the various datasets and combined these source trees via a supertree with the aid of Rainbow [27], using matrix representation with parsimony and the Baum [28] and Ragan [29] coding scheme [30,31]. The combined matrix was subjected to a parsimony analysis with the heuristic algorithm implemented in PAUP*, using 10 random

addition replicates and the tree bisection-reconnection branch-swapping algorithm [32]. Source trees were built via Bayesian analysis with MrBayes 3.1.2 [33] by running four chains of 10^6 generations. The best evolutionary models were chosen by applying a hierarchical likelihood-ratio test using MrModelTest 2.2 [34] for the rDNA sequences, and applying a mixed model to translated mtDNA sequences. The burn-in value was set to 20% of the sampled trees (1% of the number of generations). Following Loker and Brant [13], *Griphobilharzia amoena* was used as the outgroup.

Comparisons of morphological features in relation to monogamous versus polygynandrous mating system were analyzed statistically using non-parametric Mann-Whitney U-tests. We also performed variation partitioning [35,36] of these morphological features between historical (phylogeny) and potentially adaptive (mating system) components. The objective of this analysis is to estimate the fraction of the variation linked to the mating system (the potentially adaptive component), the fraction linked to phylogeny (the historical component), and the fraction linked to both phylogeny and the mating system (the overlap between the two components) for each morphological trait examined. This partitioning technique allows the user to compute the fraction of the variation of the response variable due to each explanatory trait under study (here, mating system and phylogenetic effects) while controlling other(s). This leads to “pure” fractions (here, fractions explained only by the mating system or only by the phylogeny), as well as a common fraction of the variation due simultaneously to both independent traits. We stress that this common fraction (here, the joint variation explained by mating system and phylogeny) is not equivalent to an interaction term in an analysis of variance. This overlap is usually considered to be phylogenetic niche conservatism (sensu Grafen [37]), reflecting the fact that the putative effect of the mating system on morphological features is intermingled with phylogenetic effects if species with the same mating system are closely related. Such variation in decomposition requires the quantification of trait variation due to phylogeny alone. This precludes the use of classical comparative methods, such as independent contrasts [38,39], because such methods cannot quantify phylogenetic inertia per se (see [40]). Here, the expression of the phylogenetic variance is carried out via a principal coordinate analysis on the distance matrix computed from the phylogenetic tree of the species considered. A few principal coordinates were chosen using a broken-stick model [41] to account for phylogeny. Details of the partitioning method used, which is based on the combination of R^2 values resulting from different regressions, can be found in Desdés et al. [35] and Cubo et al. [42]. Adjusted R^2 values,

which have been shown to be better in a variation-partitioning context, were used here [43]. Principal coordinate analyses were performed using DistPCoA [44]. Variation partitioning and tests of significance of the fractions were computed using the functions “varpart” and “anova.cca” from the “vegan” library [45] of the R statistical language (R Development Core Team 2008; *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>). All tests were performed using permutational procedures (9999 permutations/test). The mating system was coded as a binary variable (0/1). In the phylogeny obtained (see below), species were split into two clades—one containing the monogamous species, and the other containing the polygynandrous species. This design does not allow a proper test of whether a transition toward a given mating system is associated with a change in a morphological feature, because the most parsimonious explanation suggests that only one transition in mating system occurred (see [46]). We then computed the principal coordinates within each monophyletic group to test if having a certain mating system is related to modifications in given morphological features, while taking phylogeny into account.

Results

Phylogeny

The supertree analysis led to 14 equally parsimonious trees that were combined by consensus into a majority rule. The consensus was congruent with the tree obtained from phylogenetic analysis of 28S rDNA sequences. Because branch lengths were desirable for the subsequent statistical analysis, based on this phylogenetic tree, we then kept this 28S rDNA tree, where we collapsed some clades as polytomies as obtained in the supertree consensus, and added the taxa from which 28S rDNA sequences were missing (*Schistosoma guineensis*, *S. edwardiense*, *S. hippopotami*) (Figure 2). Branch lengths for these three species were estimated from the phylogenetic analysis based on CO1 mtDNA gene, and resized to be coherent with the lengths computed from the 28S rDNA analysis. This tree was used for the variation partitioning analyses.

Comparative analyses

In the “reproduction group” of features (Figure 3A), males in monogamous species possessed fewer testes, showed lower relative surface areas of both testes and seminal vesicles, but had higher relative gynecophoral canal lengths than did males of polygynandrous species. Both the variation-partitioning mating system and phylogenetic analyses showed that all of relative testis surface area, testis number, and relative gynecophoral canal

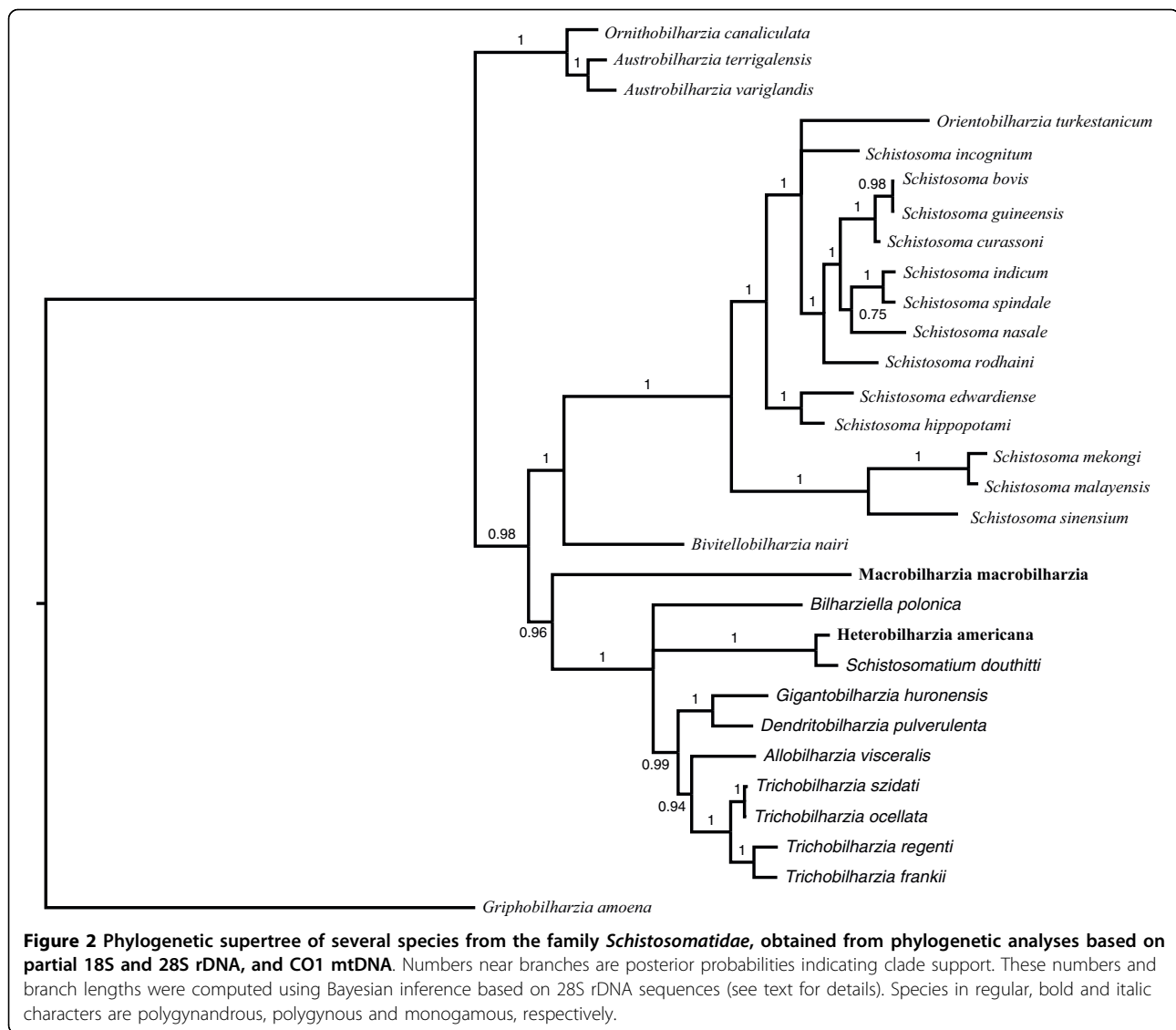
length, were significantly linked to the mating system, with R^2 values greater than 0.4 (*i.e.*, explaining more than 40% of the variance). Females of monogamous species displayed relatively lower seminal receptacle surface areas than did polygynandrous females. However, no significant association was found between this variable and the chosen mating system. Similarly, no difference in relative ovary length among females differing in mating system was observed.

Turning to the “nutrition group” of features (Figure 3B), both males and females of polygynandrous species displayed longer relative oesophagus lengths than did monogamous species. Variation-partitioning analysis suggested that this morphological feature was significantly linked to the mating system, in both sexes.

In the “locomotion group” of features (Figure 3C), males of monogamous species displayed a higher relative sucker surface area than did males of polygynandrous species. There was no difference in sucker surface area between females of monogamous and polygynandrous species. Comparative analyses suggested a significant effect of mating system only on the male/female relative sucker surface area ratio. Thus, sexual dimorphism in sucker surface area was greater in monogamous than in polygynandrous species.

Discussion

It is now well established that, as a consequence of sperm competition, males displaying promiscuous sexual behaviour need to invest more energy in the reproductive organs than do monogamous males [47]. Such a link has been shown in primate, bird, rodent, amphibian, and insect species, and also between different populations of the same species [47]. In parasitic organisms, an impact of sexual selection on morphological features has been demonstrated in polygamous acanthocephalans [48]. In the cited study, it was shown that investment in testicular volume was related to the intensity of male-male competition. Our present work provides the first evidence from a parasitic organism showing that the development of sexual tissue is dependent on the mating system, with polygynandrous male schistosomes investing more energy in reproductive organs (measured by testis size) than do monogamous males. Literature reports on the link between accessory gland size and sperm competition level are few. Recently, it was shown in rodents that the masses of both the seminal vesicle and the anterior lobe of the prostate vary positively with testis weight [7]. Without controlling for phylogeny, we found a similar link between the relative testis and vesicle surface areas in males and the associated relative seminal receptacle surface area in females. Unfortunately, variation-partitioning tests did not show any effect of mating system on the sizes of these accessory

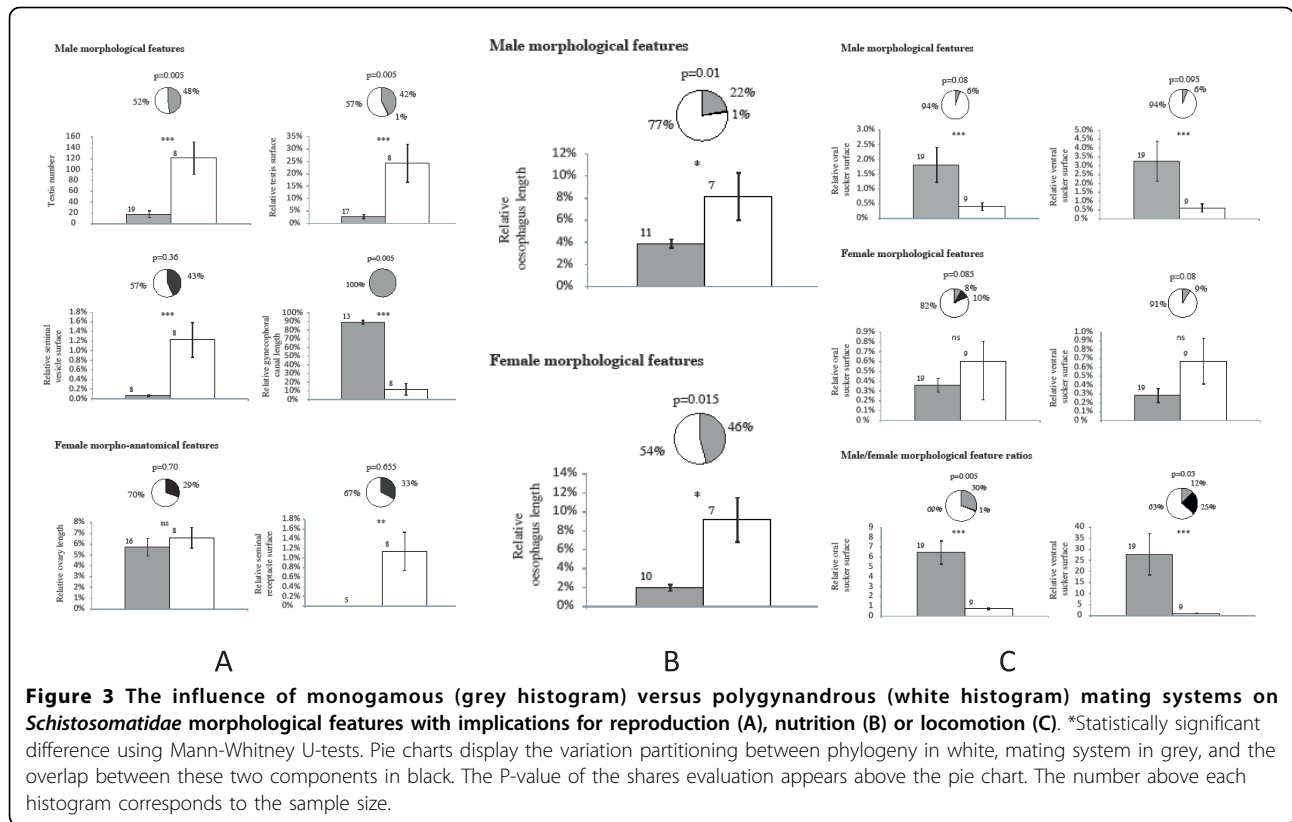


sex organs, suggesting that more species need to be included in future analysis.

The gynecophoral canal, a ventral groove in which the female resides, is a male secondary sexual characteristic specific to *Schistosomatidae*. We found that monogamous male schistosomes had gynecophoral canals 7-fold longer than those of polygynandrous males (90% vs. 12% of total body length), a difference that can be fully explained by variation in mating systems. When such systems were not considered in previous studies, a negative association was observed between the size of the gynecophoral canal and the number of testes [49]. The level of paternal investment is known to be associated with the mating system [50], and it is generally accepted that the male makes a lower investment in the system when successful paternity is less likely [51]. Thus, if the gynecophoral canal represents a paternal investment, as

has indeed been proposed [49], it seems logical that monogamous male schistosomes, which make a greater investment than do polygynandrous males, should possess longer canals.

In *Schistosomatidae*, the gonado-somatic index (*i.e.*, the relative testis surface area) ranges from 3-24% depending on whether the mating system is monogamous or polygynandrous. By comparison, testis mass as a percentage of body weight ranges from 0.12-8.4% in bats and from 0.02-0.75% in primates [3]. It might be expected that more energy is invested in testicular tissue, which is energetically demanding [52], less energy is available for other tissues and functions. The present study shows that if monogamous male *Schistosomatidae* have a lower relative testis surface area than do polygynandrous males, the relative sucker surface area is larger and the relative oesophagus length smaller.



Suckers are very important organs in digeneans because the suckers allow migration and fixation of the worm in the definitive host. In addition, because *Schistosomatidae* are endoparasites that live in the veins of birds or mammals, the organisms must be capable of resisting blood flow. Our present work showed that relative sucker dimorphism was greater in monogamous than in polygynandrous species. This difference is a consequence of a higher relative sucker surface area in monogamous males compared to polygynandrous males, rather than a variation in relative female sucker surface area. More precisely, no difference was apparent in relative sucker surface area between monogamous females and polygynandrous male or female parasites (0.41-0.58% of body surface area when both suckers were considered). Only monogamous males displayed expanded relative sucker surface areas (1.83% and 3.28% of body surface area for the oral and ventral suckers, respectively). This can be explained by the fact that, in monogamous species, the male parasite must maintain and transport its female to egg-laying sites. In contrast, females of polygynandrous species must travel and resist blood flow alone.

Schistosomes ingest red blood cells (the principal diet) using negative pressure created by contraction of the oral sucker muscle and the esophagus [53]. We found that

the oesophagus of both male and female polygynandrous parasites was longer than that of monogamous males and females. With polygynandrous males, it may be assumed that the need to produce numerous spermatozooids requires high-level nutrient intake. In addition, because such males need not hold and transport a female, the males can invest more energy in obtaining nutrition. In polygynandrous females, the longer length of the oesophagus compared to that of monogamous females may be a consequence of the absence of continuous pairing. In monogamous schistosomes (at least in the *Schistosoma* genus, for which most information is available), it is well established that the male assists the female to pump blood and to reach sexual maturity [54]. A lone female is stunted and unable to produce eggs [18]. Therefore, as a consequence of the mating system, monogamous females, aided by their males, would be expected to possess a shorter oesophagus than that of polygynandrous females, which live separately from males.

Conclusions

The present study shows that the mating system drives negative associations between somatic and sexual morphological features. Monogamous males invest less in sexual tissues (the testes and associated organs) and more in tissues required for female transport, protection,

and feeding assistance. On the other hand, polygynandrous males make a greater investment in sexual tissues and a lower investment in female care compared to monogamous males. Therefore, sexual selection acts not only on primary and secondary sex organs, but also on somatic organs, the functions of which are beneficial in a given mating system.

Additional material

Additional file 1: Table S1: Morphological features noted for each *Schistosomatidae* species. M, monogamous; P, polygynandrous; NA, no available data. The surface area of each organ was calculated based on the length (l) and the width (w) of the organ using the ellipsis surface area formula ($l \times w \pi/4$). The relative organ length is the length of the organ divided by the body length, and the relative organ surface area is the surface area of the organ divided by the body surface area.

Additional file 2: Table S1: Accession numbers of the sequences used for phylogenetic reconstruction.

Acknowledgements

We thank Serge Morand for valuable and constructive comments on the manuscript and Joan Straub for editing the English of the manuscript. This work was supported by the French Ministère de l'Enseignement Supérieur et de la Recherche, the CNRS, and the Agence Nationale de la Recherche (Program Monogamix ANR-08-BLAN-0214-02).

Author details

¹Laboratoire de Parasitologie Fonctionnelle et Evolutive, Centre de Biologie et d'Écologie Tropicale et Méditerranéenne, UMR 5244 CNRS-UPVD, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France. ²FRE 3355 CNRS-UPMC, Biologie Intégrative des Organismes Marins, Université Paris 06, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France.

Authors' contributions

JB and JP compiled the database. YD performed the phylogenetic and the comparative analyses. SB, YD and JB drafted the manuscript. All authors read and approved the final manuscript.

Received: 15 January 2010 Accepted: 10 August 2010

Published: 10 August 2010

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doi:10.1186/1471-2148-10-245

Cite this article as: Beltran *et al.*: Mating system drives negative associations between morphological features in *Schistosomatidae*. *BMC Evolutionary Biology* 2010 **10**:245.

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