

Original Article

Inbreeding depression of mating behavior
and its reproductive consequences in a
freshwater snailTim Janicke,^a Nikolas Vellnow,^a Thomas Lamy,^a Elodie Chapuis,^b and Patrice David^a^aCentre d'Ecologie Fonctionnelle et Evolutive, CNRS-UMR 5175, 1919 Route de Mende, F-34293 Montpellier Cedex 05, France and ^bCentre de Biologie pour la Gestion des Populations, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus International de Baillarguet, CS 30016, F-34988 Montferrier-sur-Lez, France

Received 2 July 2013; revised 20 November 2013; accepted 26 November 2013; Advance Access publication 30 December 2013.

Theoretical work predicts that the magnitude of inbreeding depression is particularly high in traits that are closely related to fitness. Despite the extensive work on inbreeding depression of male and female reproductive performance, relatively little is known on how inbreeding impairs male and female mating behavior. We studied inbreeding depression of male and female mating behavior in the simultaneously hermaphroditic freshwater snail *Physa acuta* to test 1) whether there is inbreeding depression of mating behavior, 2) whether the potential of mate competition and mate choice has an effect on the strength of inbreeding depression, 3) whether the magnitude of inbreeding depression differs between both sex functions, and 4) how inbreeding depression of mating behavior translates into inbreeding depression of reproductive success. For this, we compared the mating behavior between selfed (inbred) and outcrossed (outbred) focal snails in a series of mating trials, in which we manipulated experimentally the potential of mate competition and mate choice. Our results provide evidence for moderate inbreeding depression of the number of copulatory encounters, the number of copulations, and the total time spent mating in both sex functions. The magnitude of inbreeding depression did not differ between the levels of competition and between both sex functions. Finally, our results suggest that inbreeding depression of mating behavior only explains a small fraction of the observed inbreeding depression of reproductive success. We discuss the implications of these findings with respect to precopulatory sexual selection and sex-specific inbreeding depression.

Key words: inbreeding depression, male–male competition, mate choice, mating success, *Physa acuta*, sexual selection.

INTRODUCTION

Inbreeding depression, the reduced vigor and fertility of offspring of related individuals, has been argued to be a potent selective agent for the evolution of reproductive morphologies, physiologies, behaviors, and mating systems (Charlesworth and Charlesworth 1987; Pusey and Wolf 1996; Keller and Waller 2002). Inbreeding impairs fitness because of an increased level of homozygosity of inbred individuals, which may lead to the expression of deleterious recessive alleles (“partial dominance hypothesis”) and/or the loss of heterozygosity at loci with heterozygote advantage (“overdominance hypothesis”; Charlesworth and Charlesworth 1999). More recent work suggests that inbreeding depression is primarily due to the expression of deleterious alleles rather than overdominance (e.g., Roff 2002; Charlesworth and Willis 2009).

Inbreeding depression has been demonstrated for a great variety of morphological and life-history traits across a broad range of animal and plant taxa (Crnokrak and Roff 1999; Keller and Waller 2002). Overall, inbreeding depression has been found to be stronger in traits that are more closely related to fitness (DeRose and Roff 1999; Van Buskirk and Willi 2006). This is thought to be due to differences in the genetic architecture between fitness and nonfitness traits with fitness traits showing usually a lower heritability but more additive and nonadditive genetic variance, including more directional dominance (reviewed in Crnokrak and Roff 1995; Merila and Sheldon 1999). In particular, fitness-related traits are assumed to be affected by a larger number of single traits (and thus a larger number of loci) so that inbreeding depression of fitness traits is generated by a combined effect of inbreeding depression of the underlying traits (DeRose and Roff 1999).

In sexually reproducing organisms, mating success is usually closely related to male (Bateman 1948; Tang-Martinez 2010) and occasionally also female fitness (Arnqvist and Nilsson 2000), so that mating success is expected to show strong inbreeding depression.

Address correspondence to T. Janicke. E-mail: janicke.tim@gmail.com.

There are at least 3 reasons why mating success can be affected by inbreeding. First, inbreeding usually reduces overall health and body condition (e.g., Townsend et al. 2010; Rantala et al. 2011), which might translate into a lowered overall mating activity of a given individual. Second, inbreeding depression might impair the attractiveness for being chosen as a mate, which is because inbred mating partners might provide less material (direct) benefits (e.g., nutrients, parental investment, and/or protection from predators; reviewed in Kempnaers 2007) and/or genetic (indirect) benefits (Reinhold 2002; Fromhage et al. 2009). And third, inbreeding might depress the ability to outcompete rivals, which will ultimately translate into a reduced mating success (e.g., Meagher et al. 2000; Hoglund et al. 2002).

Until now, relatively little effort has been made to quantify inbreeding depression of sexually selected traits and mating behavior. Studies on zebra finches *Taeniopygia guttata* and guppies *Poecilia reticulata* revealed that inbreeding impairs sexually selected traits of males and that females were more attracted to outbred males (van Oosterhout et al. 2003; Bolund et al. 2010; Zajitschek and Brooks 2010). Moreover, females of the fruit fly *Drosophila simulans* have been shown to engage in copulation more quickly with outbred than with inbred males suggesting that outbred males are more attractive mates (Okada et al. 2011). Finally, inbred males have been demonstrated to copulate less often compared with outbred males in the poeciliid fish *Heterandria formosa* (Ala-Honkola et al. 2009) and the fruit fly *Drosophila melanogaster* (Miller et al. 1993). Most of these studies are focusing exclusively on inbreeding depression of male behavioral traits, which is presumably due to the fact that selection on mating success is assumed to be stronger in males than in females as predicted by Bateman's principle (Bateman 1948; Jones and Ratterman 2009). Indeed, in species with stronger intra- and intersexual selection on males, outbred males are expected to outcompete inbred males and at the same time females might have a preference to copulate with more outbred males. Consequently, inbreeding depression of mating success might be stronger in males than in females. Nevertheless, more recent work suggests that also males allocate their reproductive resources strategically and choose among females for the most fecund mate (for reviews, see Dewsbury 1982; Wedell et al. 2002; Edward and Chapman 2011), so that inbreeding might also affect female mating success due to a lowered attractiveness of inbred individuals.

In this study, we tested for inbreeding depression of male and female mating behavior in the simultaneously hermaphroditic freshwater snail *Physa acuta* (Draparnaud 1805). Simultaneous hermaphrodites provide interesting model systems to explore and to compare effects of inbreeding on male and female reproduction because both sex functions share the same genome and are exposed to exactly the same environmental conditions within a single individual. Similar to separate-sexed organisms, sexual selection has been argued to be stronger in the male than in the female sex function (Charnov 1979; Anthes et al. 2010; but see Leonard 2005) so that inbreeding might affect the mating behavior of both sex functions differently. Previous studies on *P. acuta* revealed that mating is beneficial for both sex functions with male reproductive success being more closely related to mating success than female reproductive success (Pélissié et al. 2012). This suggests that precopulatory sexual selection operates more strongly on the male than on the female sex function. This is supported by studies indicating precopulatory female choice in response to the relatedness (Facon et al. 2006) and the mating history of the partner (Facon et al. 2007). Until now, body size is the only morphological trait

that has been argued to be under precopulatory sexual selection in *P. acuta* as inferred from positive correlations with male and female mating success (Pélissié et al. 2012). Furthermore, postcopulatory sexual selection is presumably intense in these snails. First, paternity analyses indicated high levels of multiple mating in the male and the female sex role suggesting intense sperm competition in this species (Pélissié et al. 2012). And second, there is also scope for cryptic female choice (sensu Thornhill 1983) as snails stop fertilizing their eggs with autospERM (sperm produced by themselves) soon after being given access to allosperm (sperm received from partners; David P, unpublished data), but direct evidence for recipients choosing among sperm from different sperm donors after copulating is still lacking.

Snails of *P. acuta* are capable of reproducing both uniparentally through self-fertilization and biparentally through cross-fertilization. In natural populations, selfing occurs but at relatively low rates (<10%) and has mainly been observed in the laboratory when snails had no access to mates (Jarne et al. 2000; Henry et al. 2005; David et al. 2007; Janicke et al. 2013). In the natural populations near Montpellier (France), molecular genetic diversity is consistently high (microsatellites: H_e between 0.6 and 0.8; see Escobar et al. 2008). This suggests that despite large temporal variation in population density, drift is not sufficient to remove genetic variation when facing gene flow from surrounding populations. We therefore do not expect inbreeding depression to have been effectively purged in such contexts. Accordingly, selfing in the laboratory reveals strong inbreeding depression in the female sex function and the inbreeding load has been found to vary considerably between natural populations (Escobar et al. 2008). Inbreeding has also been demonstrated to impair male reproductive success, with stronger male inbreeding depression under conditions that allow for male–male competition (Janicke et al. 2013). It has been speculated that inbreeding depression in the male function is mainly due to a lowered sperm competitiveness of inbred individuals (Janicke et al. 2013), but the extent to which inbreeding depresses male and female reproductive output via precopulatory processes has not been quantified yet.

In this study, we compared the mating behavior between selfed (inbred) and outcrossed (outbred) snails across 3 competition treatments, which differed in their potential for precopulatory sexual selection. This allowed us to test experimentally the hypotheses 1) that inbreeding affects mating behavior, 2) that the strength of inbreeding depression of the mating behavior increases with increasing opportunity for male–male competition, and 3) that the magnitude of inbreeding depression of behavioral traits differs between both sex functions as the male and the female sex functions differ in their benefit of multiple mating. In addition, we also explored 4) whether inbreeding depression of the mating behavior can explain inbreeding depression of male and female reproductive success. Given what is known on inbreeding depression and sexual selection of the studied model species, we predicted 1) that inbreeding impairs mating behavior, 2) that inbreeding depression is stronger under conditions that allow for mate choice, and 3) that the magnitude of inbreeding depression of mating behavior is stronger in the male than in the female sex function. Finally, given that reproductive success is expected to be more closely associated with mating success in the male than in the female sex function, we expected 4) that inbreeding depression of mating behavior explains to a larger extent the inbreeding depression of reproductive success in the male than in the female sex function. To our knowledge, this is the first study quantifying inbreeding depression of the mating behavior in both sex functions in a simultaneous hermaphrodite.

METHODS

Study organism

We studied the effects of inbreeding of male and female mating behavior in the simultaneously hermaphroditic freshwater snail *P. acuta* (Basommatophora). Copulations are frequent and unilateral, which means that snails adopt either the male or the female sex function when copulating. Mating involves a behavioral sequence, during which the male-acting individual crawls on the shell of the partner until it is mounted in a position that allows to insert the phallus into the partner's gonopore (Wethington and Dillon 1996). The female-acting snail can display a rejection behavior while being mounted or inseminated by the mate (Wethington and Dillon 1996; Ohbayashi-Hodoki et al. 2004; Facon et al. 2007). This is done by swinging the shell, which has been argued to prevent the male-acting snail from copulating with its female partner and reported to occur in up to 50% of all encounters (Wethington and Dillon 1996). It has been suggested that this evasive behavior allows the female-acting snail to 1) repel an inferior sperm donor and/or 2) to adopt the male role, which is expected to be the preferred sex role in *P. acuta* (Wethington and Dillon 1996; Pélissié et al. 2012). However, the adaptive significance of this rejection behavior has not been tested yet.

In the laboratory, snails are kept at 25 °C in small plastic boxes and fed with boiled lettuce, which is provided ad libitum. Under these conditions, snails mature within 6–8 weeks and adults lay a gelatinous egg capsule containing several tens of eggs every 1–2 days.

EXPERIMENTAL SETUP

Population studied and albino culture line

This experiment focused on inbreeding depression of male and female mating behavior of snails obtained from a natural population. For this, we collected 60 adult individuals (G_0) in July 2011 from a population of the river Lez (50°55'6"N, 11°34'6"E), which is located 15 km north to Montpellier, France.

We studied inbreeding depression of mating behavior and its fitness consequences under varying intensities of male–male competition. For this, we used albinotic individuals as potential mating partners in order to quantify paternity success of focal wild-type individuals when mating with and competing against albinotic individuals (analyses of inbreeding depression of male and female reproductive success using data from the same experimental setup are reported in Janicke et al. 2013). This was possible as snails of the albino culture line we used are homozygous for a recessive, Mendelian segregating allele for albinism (David P, unpublished data). This albino line has been backcrossed 3 times into a genetically outbred and diverse population (involving more than 30 unrelated individuals at each generation) and kept in high numbers since then (>100 individuals) so that all used albinotic snails have an essentially outbred genome except for the small region around the albinism locus.

We conducted a side experiment testing for potential differences in mating behavior between albinotic and wild-type snails. For this, we assigned same-aged, virgin snails to 16 groups each comprising 2 albinotic and 2 wild-type individuals. Wild-type individuals were progeny of individuals sampled from a natural population of the river Lez (see above). All individuals were color marked (paint marker 751, Edding, Japan) prior to the mating trials. We observed the mating behavior for 3 h in 350 mL glass bowls without providing

food and recorded the number of copulations of each individual. This allowed us to test whether mating was random with respect to the pigmentation of the snails. Albinotic and wild-type snails did not differ significantly in the number of copulations in the male role (albinotic: 2.25 ± 0.20 and wild-type: 2.34 ± 0.21 ; linear mixed model [LMM] including group as a random factor: $F_{1,47} = 0.09$, $P = 0.764$) or the female role (albinotic: 2.49 ± 0.28 and wild-type: 2.09 ± 0.25 ; LMM: $F_{1,47} = 0.98$, $P = 0.326$). Furthermore, the proportion of copulations between same-pigmented snails (i.e., both partners were albinotic or wild-type) within a group was 0.29 ± 0.04 and not statistically different from the expected proportion under random mating of one-third ($t_{15} = 1.21$, $P = 0.245$). Consequently, mating success of albinotic and wild-type snails is very similar and individuals do not seem to have an overall preference for mating with albinotic or wild-type individuals. Finally, given that all focal snails in our main experiment were wild-type individuals, potential biases induced by differences between wild-type and albinotic individuals are balanced across all experimental treatments.

Breeding of focal individuals

On day 1 of the experiment, we collected 60 grandparents (G_0) of focal snails in the field and brought them to the laboratory where all individuals were isolated in boxes of 100 mL. On day 3, we removed all snails from the boxes and kept all egg capsules that were laid within 48 h. After hatching, we isolated 1 hatchling (G_1) from each G_0 mother on day 27 and raised them until maturity. Thus, all 60 G_1 individuals had different mothers and were of a similar age.

The remaining part of the experiment was split into 2 blocks that were separated by 7 days. Here, we only report the time schedule for block 1. On day 55, we performed controlled mating trials in which each of 30 randomly selected G_1 individuals was allowed to copulate once in its male sex function with another randomly selected G_1 individual. Copulation attempts in which the focal G_1 individual was trying to adopt the female sex role were prevented by gently separating the 2 snails using forceps. After these controlled mating trials, each individual of a pair was isolated to produce offspring. By this, focal G_1 individuals produced selfed and therefore inbred offspring via their female sex function, whereas their mating partners produced outcrossed offspring sired via the male sex function.

Five days after hatching, 4 G_2 snails (hereafter called “focals”) were randomly selected from each G_1 focal mother and kept in isolation until maturity. Consequently, each family comprised 4 selfed and 4 outcrossed full siblings produced by G_1 snails via the female and the male sex function, respectively.

Breeding of albinotic mating partners

Parents of potential mating partners were obtained from an albino culture line (see Population studied and albino culture line). On day 59 before the start of the mating trials, we distributed 120 parental snails equally among 8 big plastic tanks (1.5 L) to let them lay eggs and removed all adult snails 3 days later. On day 5 after hatching, we transferred all produced juveniles in groups of 5 individuals to plastic boxes. Five days later we isolated all individuals to ensure that all of them remained virgin until the start of the male competition trials.

Mating trials

From day 111 to 115, we performed a series of mating trials in which each focal snail was consecutively exposed to 3 different

competition treatments, including 1) “no” competition, 2) “indirect” competition, and 3) “direct” competition. In the “no” competition treatment, the focal snail was offered 1 virgin albinotic snail as a mating partner. Hence, in this competition treatment, there was no potential for precopulatory mate–mate competition and none of the 2 individuals could choose between different mating partners. In the “indirect” competition treatment, focal snails were offered an albinotic snail as a mating partner, which was kept together with another albinotic snail prior to the mating trials. In particular, 5 days before the mating trial, we transferred 2 virgin albinotic snails in a plastic box and allowed them to mate for 48 h. Afterward we isolated both individuals and checked whether they had laid eggs indicating that they mated in their female sex function and had sperm in storage. Only albinotic individuals that had laid eggs were offered as mating partners in the mating trials of the “indirect” competition treatment. Thus, there was no potential for direct precopulatory mate–mate competition. Nevertheless, there was some potential for indirect mate choice in terms of cryptic female choice (*sensu* Thornhill 1983) and strategic sperm allocation, as albinotic mates had allosperm from another outcrossed albinotic individual in storage and might have donated sperm to an outcrossed albinotic individual prior to the mating trial. In the “direct” competition treatment, focal snails were offered 2 randomly selected, virgin albinotic snails as mating partners at the same time. Hence, in this competition treatment, there was scope for direct precopulatory mate–mate competition and mate choice.

Mating behavior of focal snails was recorded for 45 min in each competition treatment. During the observation period, pairs (“no” competition and “indirect” competition treatment) and triplets (“direct” competition treatment) were kept in 350 mL glass bowls without providing food. After the observation period, all snails of a group were transferred together to plastic boxes (200 mL) and provided with boiled lettuce *ad libitum* for additional 23 h. Therefore, each competition treatment lasted for approximately 24 h, but the mating behavior was only recorded for the first 45 min of each treatment. After these 24 h, focal snails were kept in isolation for additional 24 h before they were exposed to another competition treatment.

All focal snails were firstly exposed to the “no” competition treatment after which we balanced the sequence of the “indirect” competition and the “direct” competition treatment among selfed and outcrossed individuals of each family (i.e., 2 selfed and 2 outcrossed individuals of each family experienced first the “no” competition followed by the “indirect” competition and finally the “direct” competition treatment, whereas the other individuals of a family experienced first the “no” competition followed by the “direct” competition and finally the “indirect” competition treatment). In order to distinguish the 2 albinotic snails in the “direct” competition treatment, we marked both individually by adding a small dot of car-body paint (Motip Dupli B.V., Wolvega, The Netherlands) on their shell, which has previously been shown to have no detrimental effects on life-history traits in the studied species (Henry and Jarne 2007). For each social group of the “direct” competition treatment, we randomly defined one albinotic snail as the assigned mating partner and the other albinotic snail as the competitor. Using the average of all behavioral parameters obtained from both albinotic individuals of a given group did not affect the results qualitatively.

Quantifying male and female mating behavior

We focused on 6 behavioral traits to quantify male and female mating behavior of focal snails. First, we recorded the number of

precopulatory encounters (hereafter called “number of encounters”) in which focal snails were trying to adopt the male or the female sex role. Specifically, a male encounter was defined as the attempt of a focal snail to mount the shell of an albinotic snail, whereas a female encounter was defined as the attempt of an albinotic snail to mount on the shell of the focal snail. We need to clarify here that a male encounter can be considered as the initiation of a copulation and therefore as a behavior, which is clearly initiated by the male-acting snail. In contrast, during a female encounter, the focal individual remains mainly passive when the male-acting snail is trying to mount on the focal’s shell except in cases when the female-acting snail is trying to reject the mate. Second, we assessed for both sex functions the “number of copulations” as an estimate of mating success. Copulations were only counted if the male-acting snail was observed to insert its phallus beneath the shell of the female-acting snail at the appropriate position where the gonopore is located. Phenotypic correlations of the 2 behavioral traits revealed that the number of encounters and the number of copulations were highly positively correlated in both sex functions (Supplementary Table S1). For this reason, we computed the “copulations per encounter” as a third behavioral parameter, which reflects the number of copulations that focal snails obtained on average per encounter. Fourth, we measured the number of “rejections per encounter,” which was defined as the proportion of cases in which an encounter was accompanied with the shell-swinging behavior. This rejection behavior has to be considered as a behavioral trait of the female-acting snail. Thus, for the male-acting snail, it represents an estimate of how often the female partner was trying to prevent copulation. Fifth, we recorded the mean “copulation duration” as the average time that an individual copulated in its male or female sex role. Thus, copulation duration estimates the time that an individual invested per copulation. Finally, we defined the overall “mating effort” as the total duration that a focal individual adopted a given sex role. Note that for the “direct” competition, we only recorded the behavioral interactions between the focal and its randomly assigned mating partner (i.e., interactions with the randomly assigned competitor are not included in the analysis).

All behavioral observations were carried out by 3 observers between 9 AM and 3 PM on days 111, 113, and 115 of the experiment. Each observer monitored the mating behavior of up to 8 groups at the same time and recorded all required data using a customized software package. There was no observer effect on any behavioral traits measured.

Quantifying male and female reproductive performance

We quantified male and female reproductive success in order to explore how inbreeding depression of the mating behavior contributes to inbreeding depression of reproductive success (for a detailed analysis of inbreeding depression of male and female reproductive success, see Janicke et al. 2013). Male reproductive success was obtained as the number of offspring sired by the focal snail within a given group. For this, we kept all albinotic mating partners for 5 days after the mating trials in isolation to lay eggs. Finally, on day 12 after the last day of egg laying, we assessed male reproductive success as the number of wild-type offspring. In order to estimate female reproductive performance of focal snails, we let all focal snails lay eggs in isolation for 5 subsequent days after the mating trials. Female reproductive success was then assessed as the number of juveniles that were counted on day 15 after egg laying.

Quantifying inbreeding depression

We estimated inbreeding depression (δ) on ln-transformed data as suggested by Johnston and Schoen (1994) as

$$\delta_{\text{in}} = -\ln\left(\frac{W_i}{W_o}\right) = \ln(W_o) - \ln(W_i)$$

where W_i is the mean of the trait value of inbred individuals and W_o is the mean trait value of outbred individuals. Note that δ_{in} is not a basic log-transformation of the classical measure of inbreeding depression (i.e., $\delta = 1 - W_i/W_o$), but rather an estimate of inbreeding load (Charlesworth and Charlesworth 1987; Willis 1999). The inbreeding load B , defined as the number of haploid lethal equivalents (Morton et al. 1956), can be obtained by dividing the estimates of δ_{in} by the inbreeding coefficient f , which is 0.5 in our study.

Statistical analyses

Initially, we intended to study inbreeding depression for 30 families with each comprising 4 outbred and 4 inbred focal individuals. However, 2 G_1 individuals did not copulate in their male sex function, so that selfed and cross-fertilized eggs were only available from 28 families. In addition, selfed offspring from 2 additional G_1 individuals did not hatch (1 family) or did not reach maturity (1 family). Consequently, our final data set comprised 26 families (i.e., 11 families in block 1 and 15 families in block 2). In the course of the mating trials and the subsequent egg laying period, 2 focal individuals died and 9 escaped from their enclosure. This resulted in a total sample size of 197 focal individuals.

Statistical analysis was done in 4 steps in order to explore inbreeding depression of the 6 behavioral traits. First, we checked whether male and female behavioral traits covaried with each other using Spearman's rank-order correlations correcting for false discovery rate using the Benjamini–Hochberg method (Benjamini and Hochberg 1995). Second, we ran mixed models to test for effects of the inbreeding treatment (i.e., selfed vs. outcrossed), the competition treatment (i.e., none vs. indirect vs. direct competition), and their interaction (i.e., testing for differences in inbreeding depression between competition treatments) on the mating behavior for both sex functions separately. Models were run with inbreeding treatment, competition treatment, mating sequence (i.e., whether focals were exposed to the indirect competition treatment before being exposed to the direct competition treatment or vice versa) and their interactions as fixed factors and block, the identity of the focal snail and the identity of the family as random factors. "Family" was included in order to infer whether there was genetic variation in behavioral traits. Note that mating sequence, block, and identity of the focal individual were primarily added to the model in order to account for variation induced by these factors.

The number of copulatory encounters and the number of copulations were modeled using generalized linear mixed models (GLMMs) with a Poisson error distribution and a log-link function. The number of copulations per encounter and the number of rejections per encounter were modeled using GLMMs assuming a binomial error distribution and a logit-link function (i.e., for copulations per encounter, we used the number of encounters that led to a copulation vs. those that did not as the response variable; for the number of rejections per encounter, we used the number of rejected mounting attempts and the number of mountings that were not rejected as the response variable). We checked for overdispersion of all GLMMs by comparing the residual deviance

and degrees of freedom. Copulation duration and overall mating effort were modeled on square-root transformed data using LMMs assuming a Gaussian error distribution. Residuals of LMMs were checked visually for normality.

Third, we tested for sex-specific inbreeding depression of all behavioral traits. This was done by estimating δ and the corresponding 95% confidence intervals (CIs) for all competition treatments using bootstrapping. Specifically, we computed the mean and the 95% CIs of $\delta = 1 - W_i/W_o$ (i.e., the classic estimate of inbreeding depression; see above) based on 10 000 bootstrap replicates for male and female behavioral parameters. Additionally, we computed the pairwise difference of δ and the 95% CIs between both sexes (i.e., $\delta_{\text{female trait}} - \delta_{\text{male trait}}$) for each competition treatment to infer whether there was sex-specific inbreeding depression. Note that confidence limits of the pairwise difference that do not include 0 indicate a significant sex-specific inbreeding depression.

Fourth, we explored the fitness consequences of inbreeding depression of mating behavior. Specifically, we asked whether the behavioral parameters that show inbreeding depression were correlated with reproductive success and to which degree inbreeding depression of mating behavior can explain inbreeding depression of reproductive success. This was done by running generalized linear models (GLMs) including reproductive success as the response variable, the behavioral trait of interest as a covariate, and the inbreeding treatment as a fixed factor. In the models including the covariate (also called "extended models"), both terms were added sequentially, so that the test of the inbreeding treatment was done after correcting statistically for variation that could be explained by the covariate. In addition, we ran so-called "reference models" in which we only included the inbreeding treatment as a fixed factor. For all GLMs, we assumed a Poisson error distribution and used a log-link function, so that the model estimates of the inbreeding treatment correspond exactly to the ln-transformed estimate of inbreeding depression (δ_{in}) with ("extended model") and without ("reference model") correcting for the behavioral trait of interest. If inbreeding depression of mating behavior is responsible for inbreeding depression of reproductive success, we expect the estimate of inbreeding depression from the "extended model" to be substantially lower compared with the one obtained from the "reference model." We note that these tests on the fitness consequences of inbreeding depression of mating behavior are mainly exploratory for 3 reasons. First, mating behavior has not been recorded for the entire time period during which the snails could interact before reproductive success was measured. Second, female reproductive success was only assessed after all mating trials and not for each competition treatment separately as done for male reproductive success. And third, the tests provide an upper estimate of the extent to which inbreeding depression of mating behavior can explain inbreeding depression of reproductive success as they do not provide any information about causality.

All statistical analyses were carried out in R v.2.15.1 (R Development Core Team 2012). Mixed models were run using the lmer function of the lme4 package (Bates et al. 2012). Statistical significance of the fixed effects and the random effect family were obtained from model comparisons using likelihood ratio tests. Values are given as means \pm standard error (SE) unless otherwise stated.

RESULTS

Male and female behavioral traits showed positive intersexual correlations except copulation duration, which tended to be negatively

correlated between both sex functions (see [Supplementary Table S1](#) for other inter- and intrasexual correlations).

Inbreeding had an effect on 3 behavioral traits of the focal snails. First, there was inbreeding depression of the number of encounters in the male and the female sex function ([Figures 1a](#) and [2a](#); [Tables 1](#) and [3](#)). Second, inbreeding depressed the number of copulations in both sex functions ([Figures 1b](#) and [2b](#); [Tables 1](#) and [3](#)), but the number of copulations per encounter did not differ significantly between inbred and outbred individuals ([Tables 1](#) and [3](#)). And third, inbred individuals allocated in total less time in adopting the male and the female sex role ([Figures 1f](#) and [2f](#); [Tables 1](#) and [3](#)). Family did not explain a significant part of the variation in any behavioral trait except for the number of copulations and overall mating effort of the female sex function ([Table 2](#)) suggesting only little additive genetic variation in male and female mating behavior in the studied population.

The competition treatment had an effect on the number of encounters, the number of copulations, and the overall mating effort in both sex functions with an overall higher behavioral activity observed in the no competition treatment ([Tables 1](#) and [3](#); [Figures 1](#) and [2](#)). However, the interaction between the inbreeding treatment and the competition treatment was not statistically significant for all behavioral traits suggesting that the strength of inbreeding depression did not differ between the competition treatments ([Tables 1](#) and [3](#)).

The sex comparison of the inbreeding depression estimates suggests a tendency for inbreeding depression of the number of

encounters and the overall mating effort to be stronger in the female role, whereas inbreeding depression of the number of copulations was stronger in the male role ([Tables 1](#) and [3](#)). However, the 95% CIs of the inbreeding depression estimates computed for the male and the female sex function are largely overlapping for all behavioral parameters suggesting no significant sex-specific inbreeding depression ([Figure 3](#)). This is supported by the fact that the 95% CIs of the pairwise difference between male and female estimates of inbreeding depression included zero for all behavioral parameters except for copulation duration in the indirect competition (bootstrapped mean difference [lower, upper 95% confidence limits]: 0.58 [1.19, 0.05]). The latter was due to the fact that inbred individuals copulated on average longer in their male sex function but shorter in their female sex function than outbred individuals ([Figure 3e](#)).

All behavioral traits that showed inbreeding depression covaried positively with reproductive success depending on the sex function and the competition treatment. Specifically, male reproductive success was related to the number of copulations and overall mating effort in the direct competition treatment ([Table 3](#)), whereas female reproductive success was positively related to the number of encounters in all competition treatments, to the number of copulations in the no competition treatment, and to overall mating effort in the direct competition treatment ([Table 4](#)). However, the model estimates of the inbreeding treatment obtained from the extended models (i.e., in which we corrected statistically for the behavioral traits) were only marginally lower compared with the ones obtained from the reference

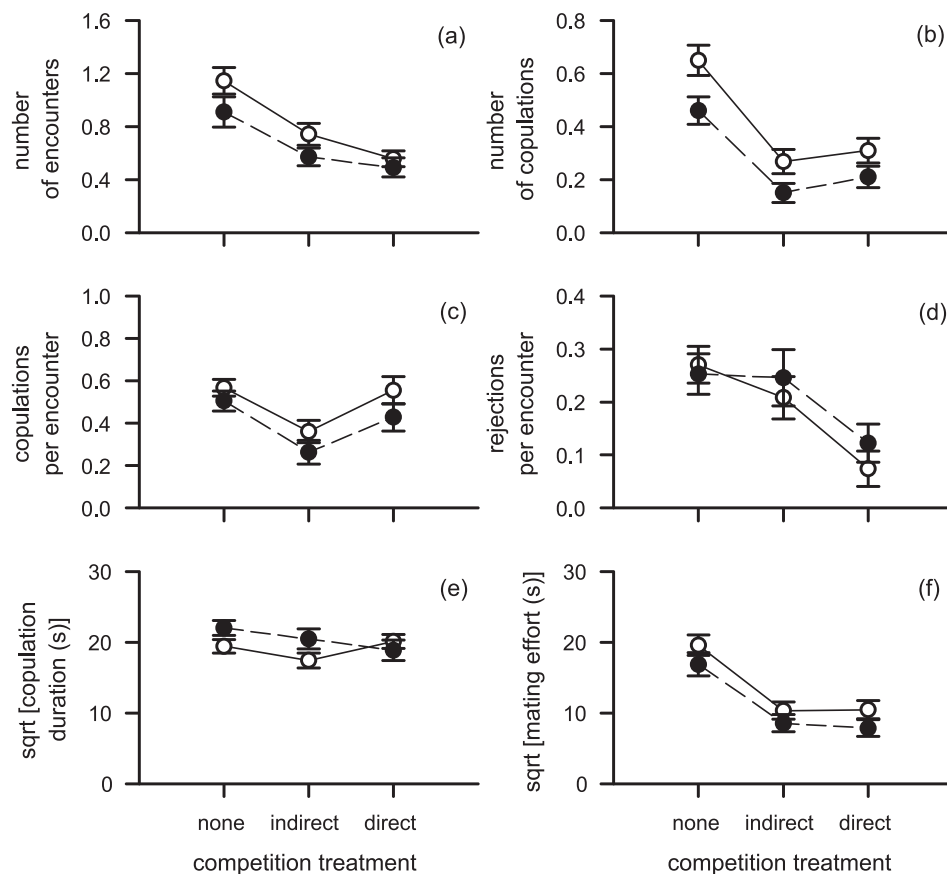


Figure 1

Comparison of male mating behavior between inbred (filled circles) and outbred (open circles) snails. Note that the number of “rejections per encounter” refers to the proportion of cases in which the mating partner was trying to reject the male-acting focal snail. Mean values \pm 1 SE are shown for (a) number of encounters, (b) number of copulations, (c) copulations per encounter, (d) rejections per encounter, (e) copulation duration and (f) mating effort across 3 competition treatments.

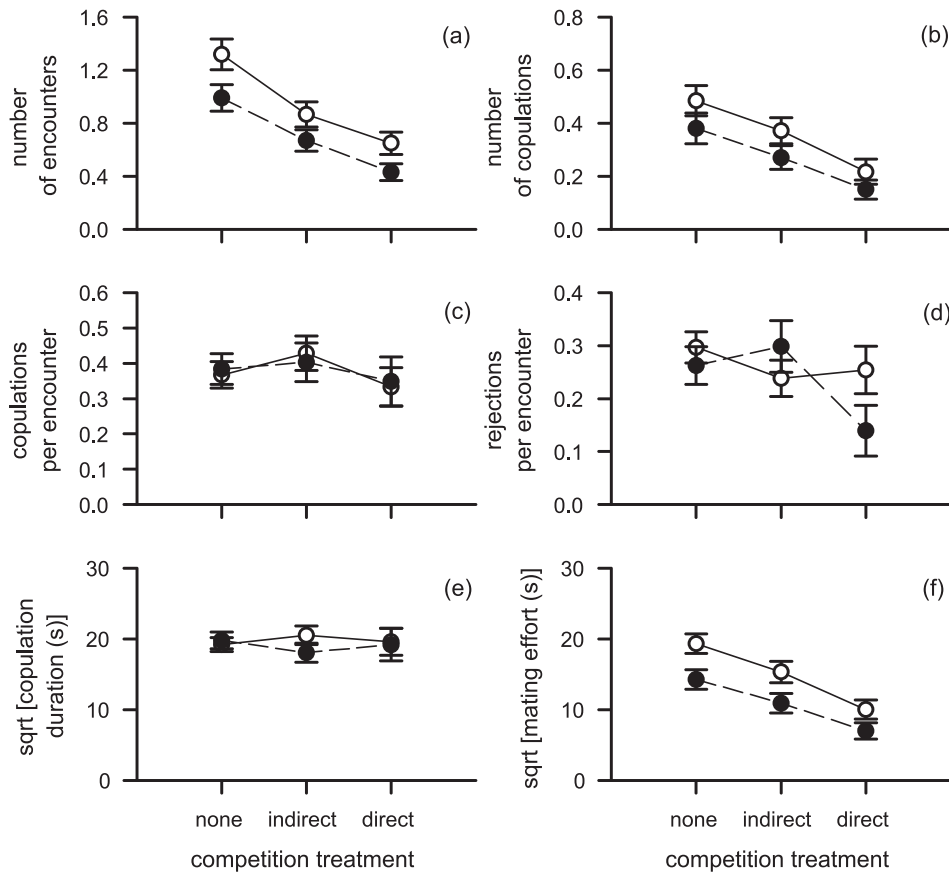


Figure 2

Comparison of female mating behavior between inbred (filled circles) and outbred (open circles) snails. Note that the number of “rejections per encounter” refers to the proportion of cases in which the female-acting snail was trying to reject the mate. Mean values \pm 1 SE are shown for (a) number of encounters, (b) number of copulations, (c) copulations per encounter, (d) rejections per encounter, (e) copulation duration, and (f) mating effort across 3 competition treatments.

models (Tables 3 and 4) suggesting that inbreeding depression of the behavioral traits does not explain a major fraction of the inbreeding depression observed for male and female reproductive success.

DISCUSSION

This study revealed inbreeding depression of the mating behavior in both sex functions of the simultaneously hermaphroditic freshwater snail *P. acuta*. We found 1) that inbred individuals showed fewer precopulatory encounters, copulated less frequently, and allocated in total less time to mating than outbred individuals; 2) that the magnitude of inbreeding depression of mating success did not depend on the potential for precopulatory sexual selection; 3) that the magnitude of inbreeding depression of mating behavior did not differ substantially between both sex functions; and 4) that inbreeding depression of the mating behavior contributes only slightly to inbreeding depression of reproductive success. We will discuss these main findings in turn.

Inbreeding depression of mating behavior

We found inbreeding depression for the number of encounters, the number of copulations, and the overall mating effort but no evidence that inbreeding impairs the number of rejections per encounter and the average copulation duration. Furthermore, there was a tendency in all competition treatments for inbred individuals

to show a reduced number of male copulations per encounter than outbred individuals, but this effect was not statistically significant. Our estimates of the magnitude of inbreeding depression (expressed as the genetic load B) that we obtained for the number of copulatory encounters (i.e., 0.44 and 0.60 for the male and the female sex function, respectively), the number of copulations (i.e., 0.80 and 0.58 for the male and the female sex function, respectively), and the overall mating effort (i.e., 0.26 and 0.78 for the male and the female sex function, respectively) are relatively moderate compared with what has been observed in other organisms. For instance, in the fruit fly *D. melanogaster*, the genetic load for the number of copulatory encounters and the proportion of copulating males have been found to range from 0.00 to 0.36 and from -0.15 to 3.10, respectively (Miller et al. 1993). Furthermore, a study on the African Butterfly *Bicyclus anynana* found a genetic load of male mating success in captivity of up to 1.5 and in free flight experiments of even up to 3.37 (Joron and Brakefield 2003). Finally, males of the Least Killifish *H. formosa* derived from one generation of sib-mating showed a lower frequency of copulatory attempts (i.e., measured as gonopodial activity) compared with outbred males with a genetic load of 3.8 (Ala-Honkola et al. 2009). However, inbreeding was also found to have no effect on mating success in the flour beetle *Tribolium castaneum* (Michalczyk et al. 2010). Nevertheless, inbreeding depression of mating behavior in *P. acuta* seems to be low compared with most other organisms.

Table 1
Estimates of male inbreeding depression (a) and results of GLMMs (b) testing the effect of the inbreeding treatment, the competition treatment, the mating sequence, their interactions, and the family on male behavioral traits

Predictor	Number of encounters		Number of copulations		Copulations per encounter		Rejections per encounter		Copulation duration (s)		Mating effort (s)	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
(a) Inbreeding												
Outbred individuals	291	0.81 ± 0.05	291	0.41 ± 0.03	179	0.50 ± 0.03	179	0.21 ± 0.02	115	409.30 ± 26.01	291	371.88 ± 29.06
Inbred individuals	300	0.66 ± 0.05	300	0.27 ± 0.03	148	0.42 ± 0.03	148	0.22 ± 0.03	81	481.64 ± 35.40	300	325.00 ± 30.95
δ_{in}	591	0.22	591	0.40	327	0.19	327	-0.05	196	-0.16	591	0.13
(b) GLMM												
Predictor	df	χ^2	df	χ^2	df	χ^2	df	χ^2	df	χ^2	df	χ^2
Inbreeding (ID)	1	4.61*	1	8.21**	1	2.71	1	0.01	1	2.30	1	3.88*
Competition (COMP)	2	35.32***	2	37.99***	2	18.41***	2	10.80**	2	2.44	2	64.08***
Sequence (SEQ)	1	4.86*	1	1.16	1	0.08	1	3.38	1	0.77	1	0.49
Family	1	0.17	1	0.00	1	3.04	1	3.26	1	0.33	1	1.32
ID × COMP	2	0.28	2	0.40	2	1.09	2	1.41	2	3.16	2	0.18
ID × SEQ	1	0.29	1	0.35	1	1.36	1	5.17*	1	0.44	1	0.21
COMP × SEQ	2	3.31	2	1.85	2	1.32	2	2.72	2	0.27	2	1.89
ID × COMP × SEQ	7	5.31	7	6.08	7	6.08	7	11.14	7	6.45	7	5.38

χ^2 values and the corresponding *P* values were obtained from likelihood ratio tests (see Methods for details). df, degrees of freedom. ****P* < 0.001, ***P* < 0.01, and **P* < 0.05.

Table 2
Estimates of female inbreeding depression (a) and results of GLMMs (b) testing the effect of the inbreeding treatment, the competition treatment, the mating sequence, their interactions, and the family on female behavioral traits

Predictor	Number of encounters		Number of copulations		Copulations per encounter		Rejections per encounter		Copulation duration (s)		Mating effort (s)	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE
(a) Inbreeding												
Outbred individuals	291	0.95 ± 0.06	291	0.36 ± 0.03	184	0.38 ± 0.03	184	0.27 ± 0.02	99	442.80 ± 30.74	291	430.13 ± 31.04
Inbred individuals	300	0.70 ± 0.05	300	0.27 ± 0.03	154	0.38 ± 0.03	154	0.25 ± 0.03	75	414.28 ± 37.81	300	292.19 ± 26.77
δ_{in}	591	0.30	591	0.29	338	-0.01	338	0.08	174	0.07	591	0.39
(b) GLMM												
Predictor	df	χ^2	df	χ^2	df	χ^2	df	χ^2	df	χ^2	df	χ^2
Inbreeding (ID)	1	10.28**	1	3.99*	1	0.08	1	0.17	1	0.49	1	12.04***
Competition (COMP)	2	45.99***	2	20.49***	2	4.02	2	1.95	2	0.01	2	42.37***
Sequence (SEQ)	1	2.52	1	3.00	1	1.02	1	0.16	1	0.08	1	3.63
Family	1	0.41	1	4.26*	1	3.46	1	3.68	1	1.30	1	6.31*
ID × COMP	2	0.40	2	0.11	2	0.20	2	3.57	2	1.71	2	0.72
ID × SEQ	1	1.28	1	1.45	1	0.31	1	0.86	1	5.42*	1	3.67
COMP × SEQ	2	2.93	2	5.09	2	2.80	2	1.25	2	1.08	2	3.36
ID × COMP × SEQ	7	6.39	7	6.76	7	3.44	7	7.57	7	9.81	7	9.20

χ^2 values and the corresponding *P* values were obtained from likelihood ratio tests (see Methods for details). df, degrees of freedom. ****P* < 0.001, ***P* < 0.01, and **P* < 0.05.

Table 3
Effect of male mating behavior on male reproductive success

Competition	Model	Covariate effect				Inbreeding effect				
		Estimate SE	df _{num}	df _{denom}	F-value	Estimate SE	Relative difference ^a	df _{num}	df _{denom}	F-value
None	Reference model	—	—	—	—	0.22 ± 0.22	—	1	192	2.98
	Encounters	0.08 ± 0.05	1	192	2.69	0.20 ± 0.13	0.08	1	191	2.51
	Copulations	0.07 ± 0.12	1	192	0.80	0.21 ± 0.13	0.06	1	191	2.49
	Mating effort	0.00002 ± 0.0001	1	192	0.07	0.22 ± 0.13	0.00	1	191	2.93
Indirect	Reference model	—	—	—	—	1.52 ± 0.35	—	1	192	24.62***
	Encounters	-0.14 ± 0.18	1	192	0.08	1.55 ± 0.35	-0.01	1	191	25.19***
	Copulations	-0.15 ± 0.33	1	192	0.05	1.54 ± 0.35	-0.01	1	191	24.74***
	Mating effort	-0.0002 ± 0.0004	1	192	0.13	1.53 ± 0.35	-0.01	1	191	24.76***
Direct	Reference model	—	—	—	—	0.72 ± 0.26	—	1	193	7.83**
	Encounters	0.29 ± 0.18	1	193	2.66	0.71 ± 0.26	0.01	1	192	7.83**
	Copulations	0.67 ± 0.25	1	193	8.04**	0.65 ± 0.27	0.10	1	192	6.29*
	Mating effort	0.0008 ± 0.0003	1	193	7.96**	0.68 ± 0.26	0.06	1	192	7.28**

The number of encounters (extended model “encounters”), the number of copulations (extended model “copulations”), and the mating effort (extended model “mating effort”) were added as covariates to a reference GLM (including only the inbreeding treatment). Model estimates of the inbreeding treatment correspond to ln-transformed estimates of inbreeding depression (δ_{in}). df_{num} and df_{denom} refer to the numerator degrees of freedom and the denominator degrees of freedom, respectively.

^aDifference between model estimates obtained from the reference model and the extended model given as percent.

*** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$.

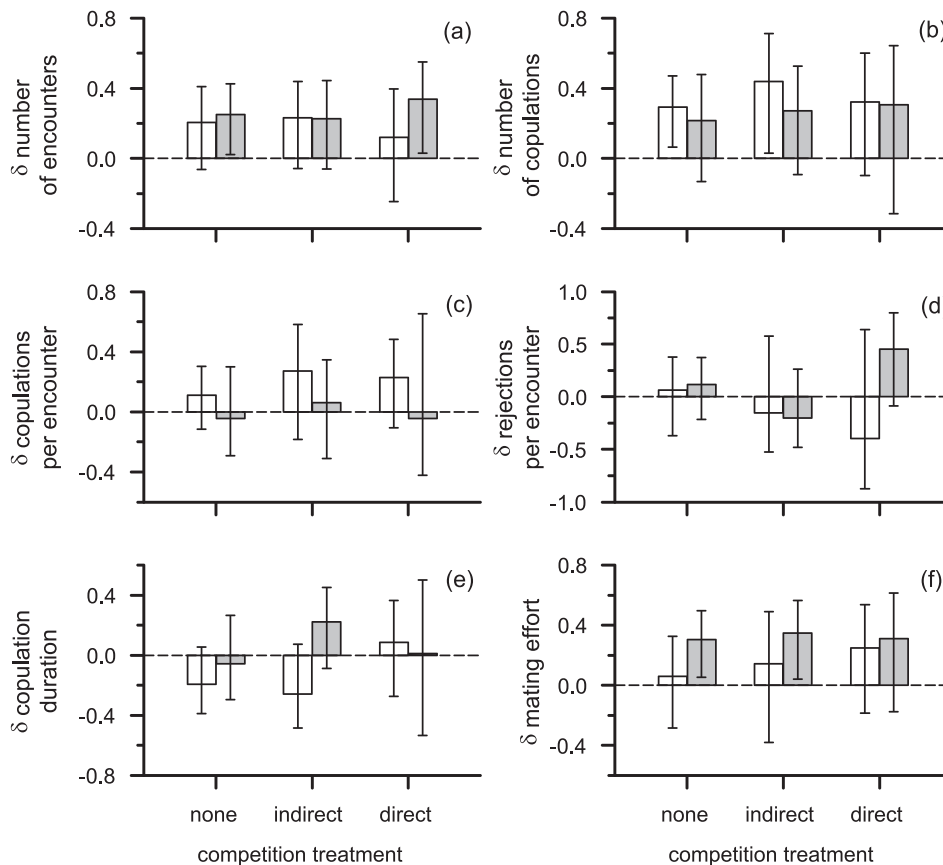


Figure 3

Comparison of the magnitude of inbreeding depression of mating behavior between the male (white bars) and the female (gray bars) sex function. Mean estimates of inbreeding depression ($\delta = 1 - W_{inbred}/W_{outbred}$) and their 95% confidence limits (obtained from bootstrapping) are shown for (a) number of encounters, (b) number of copulations, (c) copulations per encounter, (d) rejections per encounter, (e) copulation duration, and (f) mating effort separately for each competition treatment (see Methods for details).

Table 4
Effect of female mating behavior on female reproductive success

Competition	Model	Covariate effect				Inbreeding effect				
		Estimate SE	df _{num}	df _{denom}	F-value	Estimate SE	Relative difference ^a	df _{num}	df _{denom}	F-value
Overall	Reference model	—	—	—	—	0.64 ± 0.13	—	1	195	26.61***
None	Encounters	0.12 ± 0.05	1	195	9.28**	0.60 ± 0.13	0.06	1	194	23.46***
	Copulations	0.19 ± 0.10	1	195	4.88*	0.62 ± 0.13	0.03	1	194	25.13***
	Mating effort	0.0001 ± 0.0001	1	195	3.89	0.62 ± 0.13	0.04	1	194	24.17***
Indirect	Encounters	0.10 ± 0.06	1	195	4.71*	0.62 ± 0.13	0.03	1	194	24.79***
	Copulations	-0.19 ± 0.13	1	195	0.71	0.66 ± 0.13	-0.03	1	194	27.98***
	Mating effort	-0.0001 ± 0.0001	1	195	0.09	0.66 ± 0.13	-0.03	1	194	27.69***
Direct	Encounters	0.12 ± 0.08	1	195	5.63*	0.61 ± 0.13	0.05	1	194	23.58***
	Copulations	0.20 ± 0.13	1	195	3.56	0.63 ± 0.13	0.02	1	194	25.28***
	Mating effort	0.00024 ± 0.00012	1	195	5.61*	0.62 ± 0.13	0.03	1	194	24.74***

The number of encounters (extended model “encounters”), the number of copulations (extended model “copulations”), and the mating effort (extended model “mating effort”) were added as covariates to a reference GLM (including only the inbreeding treatment). Model estimates of the inbreeding treatment correspond to ln-transformed estimates of inbreeding depression (δ_{in}). df_{num} and df_{denom} refer to the numerator degrees of freedom and the denominator degrees of freedom, respectively.

^aDifference between model estimates obtained from the reference model and the extended model given as percent.

*** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$.

There are at least 3 explanations for why inbred snails might have displayed lowered mating activity compared with outbred individuals. First, inbreeding might have impaired overall locomotor activity leading to fewer copulatory encounters, fewer copulations, and an overall lowered time allocation toward mating. Second, inbred individuals might have been less attractive mates, thus reducing the motivation of other snails to mate with them. And third, inbred individuals might have been less motivated to search for a mate and to copulate due to a lowered sexual activity (e.g., due to a reduced hormonal production), which would also translate into a lowered number of precopulatory encounters and copulations. Based on our data, we cannot exclude one of these mutually nonexclusive hypotheses. However, we found an only weak and statistically nonsignificant effect of the inbreeding treatment on the number of copulations per encounters in the male sex role, which suggests that the inbreeding status of a focal has only little influence on whether a copulatory encounter results in a copulation or not. This is supported by our findings on the number of rejections per encounter indicating that once mounted on their partner’s shell, inbred individuals are not rejected (through shell-swinging behavior) more often than outbred individuals. Consequently, precopulatory mate choice against inbred individuals, if it exists, has to take place at a very early episode of the mating sequence. For instance, mate choice decisions might be based on stimuli received at the first physical contact with the mate or during mate searching based on cues associated with mucus trails as has been shown in other gastropods (e.g., Johannesson et al. 2008).

Precopulatory avoidance of inbred mates has been found in several separate-sexed organisms such as guppies (Zajitschek and Brooks 2010; but see Michalczuk et al. 2010). Furthermore, there is evidence for mate choice against inbred individuals in the simultaneously hermaphroditic cestode *Schistocephalus solidus*. In particular, mate choice experiments in this reciprocally copulating hermaphrodite suggest that outbred but not inbred focal worms have a preference to mate with outbred individuals (Schjorring 2009). Until now, mate choice experiments in our model organism *P. acuta* have focused only on effects of the relatedness (Facon et al. 2006) and the mating history (Wethington and Dillon 1996; Facon et al. 2007) of the mating partner. Based on our findings, it is difficult to infer whether inbreeding impairs the attractiveness and thereby affects precopulatory mate choice.

Further experiments in which a focal individual can choose between inbred and outbred mating partners at the same time are required to evaluate the impact of mate choice for the observed inbreeding depression of mating behavior in *P. acuta*.

Effects of competition and sex on inbreeding depression

The magnitude of inbreeding depression of all behavioral traits did not differ between the 3 competition treatments as indicated by nonsignificant interactions between the inbreeding treatment and the competition treatment. Therefore, the potential for mate competition and mate choice does not have an effect on the strength of inbreeding depression of copulatory behavior in both sex functions. This is surprising as we expected inbreeding depression to be stronger in the direct competition treatment due to a higher potential for mate competition and mate choice. For instance, male–male competition has been found to intensify inbreeding depression in the House Mouse *Mus musculus*, which could partly be explained by a reduced ability of inbred individuals to establish territories under competition (Meagher et al. 2000). In this study, we found no effect of the competition treatment on inbreeding depression of the mating behavior, which suggests that inbreeding depression is not magnified under conditions that allow for more precopulatory mate competition and mate choice in *P. acuta*.

Our results also demonstrate that the magnitude of inbreeding depression of mating success did not differ significantly between both sex functions. Despite the fact that the estimates of inbreeding depression for the number of copulatory encounters and the number of copulations tended to differ between both sex functions, the pairwise comparison of bootstrapped estimates of inbreeding depression suggests that these differences are far from statistical significance. Therefore, inbreeding seems to have a similar detrimental effect on the mating behavior in both sex functions.

Reproductive consequences of inbreeding depression of mating behavior

Inbreeding impaired male and female reproductive success of focals substantially (i.e., estimates of δ_{in} of male reproductive success for

the “no,” “indirect,” and “direct” competition treatment were 0.22, 1.52, and 0.72, respectively; δ_{in} of female reproductive success was 0.64; for details, see Janicke et al. 2013) and our results suggest that a small fraction of it can be explained by inbreeding depression of mating behavior. In particular, we found that the number of encounters, the number of copulations, and the overall mating effort had a positive effect on male and female reproductive success depending on the competition treatment. This supports an earlier finding showing that copulatory mating success is correlated with reproductive success in both sex functions in *P. acuta* (Pélissier et al. 2012). As a consequence of such a positive relationship between mating behavior and reproductive success, the obtained estimates of male and female inbreeding depression of reproductive success were in general lower when correcting statistically for the behavioral traits that showed inbreeding depression. However, inbreeding depression of reproductive success was only lowered by at most 10% in the male sex function (when accounting for the number of copulations) and by at most 6% in the female sex function (when accounting for the overall mating effort), which suggests that inbreeding depression of mating behavior can only explain a small fraction of the observed inbreeding depression of reproductive success. Therefore, we suspect that inbreeding depression of male reproductive success is mainly caused by inbreeding depression of traits that affect the outcome of postcopulatory episodes of sexual selection, whereas inbreeding depression of female reproductive success is mainly the result of a reduced fecundity (e.g., lowered egg production and/or hatching success; see also Escobar et al. 2008; Janicke et al. 2013) rather than a lowered mating success of inbred individuals. The hypothesis that inbreeding depression impairs male reproductive performance primarily at the postcopulatory stage is also supported by the fact that we found no evidence for competition-specific inbreeding depression of mating behavior despite the fact that inbreeding depression of male reproductive success differed substantially between the competition treatments with the strongest depression detected in the “indirect” competition treatment (Janicke et al. 2013).

Finally, our results also suggest that inbred individuals gain relatively less reproductive success per investment into mating than outbred individuals. This is because male mating effort (i.e., the total time spent in the male sex role) can be considered as an estimate of male allocation and was found to decrease on average by 12.6% due to inbreeding. This stays in contrast to the much stronger reduction of male reproductive success in inbred individuals of 19.7%, 78.1%, and 51.3% for the “no,” “indirect,” and the “direct” competition treatment, respectively (Janicke et al. 2013). Consequently, inbreeding depresses the fitness gain per unit of male mating effort.

CONCLUSIONS

In this study, we found evidence for moderate inbreeding depression of male and female mating behavior in the simultaneously hermaphroditic freshwater snail *P. acuta*. The strength of inbreeding depression did not differ between both sex functions and was not affected by the potential for precopulatory mate competition and mate choice. Together with earlier findings on reproductive success, we could show that inbreeding depression of the mating behavior contributes only little to the inbreeding depression of reproductive success suggesting that inbreeding impairs primarily traits that affect the outcome of postcopulatory sexual selection. Further studies are needed to identify the underlying processes that lead to the observed inbreeding depression of the mating behavior and reproductive success.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

FUNDING

This study was funded by postdoctoral fellowships from the Swiss National Science Foundation to T.J. (PBBSP3-135985 and PA00P3-145375/1) as well as funds from the “Chercheurs(euses) d’avenir” program (Région Languedoc-Roussillon) and Bio-Adapt program (ANR ESHAP) to P.D.

The authors wish to thank V. Sarda for technical support. We are also grateful to the editor and 2 anonymous reviewers for constructive comments on a previous draft.

Handling editor: Alexei Maklakov

REFERENCES

- Ala-Honkola O, Uddström A, Pauli BD, Lindström K. 2009. Strong inbreeding depression in male mating behaviour in a poeciliid fish. *J Evol Biol.* 22:1396–1406.
- Anthes N, David P, Auld JR, Hoffer JN, Jarne P, Koene JM, Kokko H, Lorenzi CM, Pélissier B, Sprenger D, et al. 2010. Bateman gradients in hermaphrodites: an extended approach to quantify sexual selection. *Am Nat.* 176:249–263.
- Arnqvist G, Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav.* 60:145–164.
- Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity.* 2:349–368.
- Bates D, Maechler M, Bolker B. 2012. lme4: linear mixed-effects models using Eigen and Eigen. R package, version 0.999999-0. Available from: <http://lme4.r-forge.r-project.org/>.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Stat Methodol.* 57:289–300.
- Bolund E, Martin K, Kempnaers B, Forstmeier W. 2010. Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Anim Behav.* 79:947–955.
- Charlesworth B, Charlesworth D. 1999. The genetic basis of inbreeding depression. *Genet Res.* 74:329–340.
- Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst.* 18:237–268.
- Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. *Nat Rev Genet.* 10:783–796.
- Charnov EL. 1979. Simultaneous hermaphroditism and sexual selection. *Proc Natl Acad Sci USA.* 76:2480–2484.
- Crnokrak P, Roff DA. 1995. Dominance variance—associations with selection and fitness. *Heredity.* 75:530–540.
- Crnokrak P, Roff DA. 1999. Inbreeding depression in the wild. *Heredity.* 83:260–270.
- David P, Pujol B, Viard F, Castella V, Goudet J. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Mol Ecol.* 16:2474–2487.
- DeRose MA, Roff DA. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution.* 53:1288–1292.
- Dewsbury DA. 1982. Ejaculate cost and male choice. *Am Nat.* 119:601–610.
- Draparnaud J-P-R. 1805. *Historie naturelle des mollusques terrestres et fluviatiles de la France*. Ouvrage posthume. Paris: Louis Colas, Libraire.
- Edward DA, Chapman T. 2011. The evolution and significance of male mate choice. *Trends Ecol Evol.* 26:647–654.
- Escobar JS, Nicot A, David P. 2008. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics.* 180:1593–1608.
- Facon B, Ravigne V, Goudet J. 2006. Experimental evidence of inbreeding avoidance in the hermaphroditic snail *Physa acuta*. *Evol Ecol.* 20:395–406.
- Facon B, Ravigne V, Sauteur L, Goudet J. 2007. Effect of mating history on gender preference in the hermaphroditic snail *Physa acuta*. *Anim Behav.* 74:1455–1461.
- Fromhage L, Kokko H, Reid JM. 2009. Evolution of mate choice for genome-wide heterozygosity. *Evolution.* 63:684–694.

- Henry PY, Bousset L, Sourrouille P, Jarne P. 2005. Partial selfing, ecological disturbance and reproductive assurance in an invasive freshwater snail. *Heredity* (Edinb). 95:428–436.
- Henry PY, Jarne P. 2007. Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. *Invertebr Biol*. 126:138–153.
- Hoglund J, Piernney SB, Alatalo RV, Lindell J, Lundberg A, Rintamaki PT. 2002. Inbreeding depression and male fitness in black grouse. *Proc R Soc B Biol Sci*. 269:711–715.
- Janicke T, Vellnow N, Sarda V, David P. 2013. Sex-specific inbreeding depression depends on the strength of male-male competition. *Evolution*. 67:2861–2875.
- Jarne P, Perdieu MA, Pernot AF, Delay B, David P. 2000. The influence of self-fertilization and grouping on fitness attributes in the freshwater snail *Physa acuta*: population and individual inbreeding depression. *J Evol Biol*. 13:645–655.
- Johannesson K, Havenhand JN, Jonsson PR, Lindegarth M, Sundin A, Hollander J. 2008. Male discrimination of female mucous trails permits assortative mating in a marine snail species. *Evolution*. 62:3178–3184.
- Johnston MO, Schoen DJ. 1994. On the measurement of inbreeding depression. *Evolution*. 48:1735–1741.
- Jones AG, Ratterman NL. 2009. Mate choice and sexual selection: what have we learned since Darwin? *Proc Natl Acad Sci USA*. 106:10001–10008.
- Joron M, Brakefield PM. 2003. Captivity masks inbreeding effects on male mating success in butterflies. *Nature*. 424:191–194.
- Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol*. 17:230–241.
- Kempnaers B. 2007. Mate choice and genetic quality: a review of the heterozygosity theory. In: Brockmann HJ, Roper TJ, Naguib M, Wynne-Edwards KE, Barnard C, Mitani J, editors. *Advances in the study of behavior*. Vol. 37. San Diego (CA): Elsevier Academic Press Inc. p. 189–278.
- Leonard JL. 2005. Bateman's principle and simultaneous hermaphrodites: a paradox. *Integr Comp Biol*. 45:856–873.
- Meagher S, Penn DJ, Potts WK. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proc Natl Acad Sci USA*. 97:3324–3329.
- Merila J, Sheldon BC. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity*. 83:103–109.
- Michalczyk L, Martin OY, Millard AL, Emerson BC, Gage MJ. 2010. Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. *Proc Biol Sci*. 277:3483–3491.
- Miller PS, Glasner J, Hedrick PW. 1993. Inbreeding depression and male-mating behavior in *Drosophila melanogaster*. *Genetica*. 88:29–36.
- Morton NE, Crow JF, Muller HJ. 1956. An estimate of the mutational damage in man from data on consanguineous marriages. *Proc Natl Acad Sci USA*. 42:855–863.
- Ohbayashi-Hodoki K, Ishihama F, Shimada M. 2004. Body size-dependent gender role in a simultaneous hermaphrodite freshwater snail, *Physa acuta*. *Behav Ecol*. 15:976–981.
- Okada K, Blount JD, Sharma MD, Snook RR, Hosken DJ. 2011. Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in *Drosophila simulans*. *J Evol Biol*. 24:363–371.
- van Oosterhout C, Trigg RE, Carvalho GR, Magurran AE, Hauser L, Shaw PW. 2003. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. *J Evol Biol*. 16:273–281.
- Pélissié B, Jarne P, David P. 2012. Sexual selection without sexual dimorphism: Bateman gradients in a simultaneous hermaphrodite. *Evolution*. 66:66–81.
- Pusey A, Wolf M. 1996. Inbreeding avoidance in animals. *Trends Ecol Evol*. 11:201–206.
- R Development Core Team. 2012. R: a language and environment for statistical computing, 2.15.2. Vienna (Austria): R Foundation for Statistical Computing.
- Rantala MJ, Viitaniemi H, Roff DA. 2011. Effects of inbreeding on potential and realized immune responses in *Tenebrio molitor*. *Parasitology*. 138:906–912.
- Reinhold K. 2002. Modelling the evolution of female choice strategies under inbreeding conditions. *Genetica*. 116:189–195.
- Roff DA. 2002. Inbreeding depression: tests of the overdominance and partial dominance hypotheses. *Evolution*. 56:768–775.
- Schjorring S. 2009. Sex allocation and mate choice of selfed and outcrossed *Schistocephalus solidus* (Cestoda). *Behav Ecol*. 20:644–650.
- Tang-Martinez Z. 2010. Bateman's principles: original experiment and modern data for and against. In: Breed MD, Moore J, editors. *Encyclopedia of animal behavior*. Oxford: Academic Press. p. 166–176.
- Thornhill R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am Nat*. 122:765–788.
- Townsend AK, Clark AB, McGowan KJ, Miller AD, Buckles EL. 2010. Condition, innate immunity and disease mortality of inbred crows. *Proc Biol Sci*. 277:2875–2883.
- Van Buskirk J, Willi Y. 2006. The change in quantitative genetic variation with inbreeding. *Evolution*. 60:2428–2434.
- Wedell N, Gage MJG, Parker GA. 2002. Sperm competition, male prudence and sperm-limited females. *Trends Ecol Evol*. 17:313–320.
- Wethington AR, Dillon RT. 1996. Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*. *Anim Behav*. 51:1107–1118.
- Willis JH. 1999. Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. *Genetics*. 153:1885–1898.
- Zajitschek SRK, Brooks RC. 2010. Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata*. *Behav Ecol*. 21:884–891.