

PATTERNS OF MATING-SYSTEM EVOLUTION IN HERMAPHRODITIC ANIMALS: CORRELATIONS AMONG SELFING RATE, INBREEDING DEPRESSION, AND THE TIMING OF REPRODUCTION

Juan S. Escobar,^{1,2,3} Josh R. Auld,^{4,5} Ana C. Correa,^{6,7} Juan M. Alonso,⁸ Yves K. Bony,^{8,9}

Marie-Agnès Coutellec,¹⁰ Joris M. Koene,¹¹ Jean-Pierre Pointier,⁸ Philippe Jarne,¹ and Patrice David¹

¹Centre d'Ecologie Fonctionnelle et Evolutive UMR 5175, 1919 Route de Mende, Campus CNRS, 34293 Montpellier Cedex 5, France

²E-mail: jsescobar2002@yahoo.fr

⁴University of Pittsburgh, Department of Biological Sciences, 101 Clapp Hall, 4249 Fifth Avenue, Pittsburgh, Pennsylvania 15260

⁶Institut de Recherche pour le Développement, GEMI UMR 2724 CNRS-IRD, 911 Avenue Agropolis, 34394 Montpellier, France

⁷Programa de Estudio y Control de Enfermedades Tropicales, Universidad de Antioquia, Calle 62 N° 52–59, Sede de Investigación Universitaria, Medellín, Colombia

⁸USR 3278 CNRS-EPHE CRIOBE, Université de Perpignan, 68860 Perpignan cedex, France

⁹Department of Biological Sciences, Laboratoire d'Environnement et de Biologie Aquatique (LEBA), URES de Daloa (University of Abobo-Adjamé), 28 BP 465, Abidjan 28, Ivory Coast

¹⁰Institut National de Recherche Agronomique, UMR0985 Ecologie et Santé des Ecosystèmes, 65 rue de Saint-Brieuc, CS 84215, F-35042 Rennes Cedex, France

¹¹Animal Ecology, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

Received June 11, 2010

Accepted December 9, 2010

In hermaphrodites, traits that influence the selfing rate can coevolve with inbreeding depression, leading to the emergence of evolutionary syndromes. Theory predicts a negative correlation between inbreeding depression and selfing rate across species. This prediction has only been examined and validated in vascular plants. Furthermore, selfing rates are often influenced by environmental conditions (e.g., lack of mates or pollinators), and species are predicted to evolve mechanisms to buffer this variation.

³Present address: Institut des Sciences de l'Evolution UMR 5554, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France.

⁵Present address: National Evolutionary Synthesis Center (NESCent), 2024 W. Main St., Suite A200, Durham, NCS 27705.

We extend previous studies of mating-system syndromes in two ways. First, we assembled a new dataset on Basommatophoran snails (17 species, including new data on 12 species). Second, we measured how species responded to variation in mate availability. Specifically, we quantified the waiting time before selfing (i.e., how long the onset of reproduction is delayed in the absence of mates). Selfing rates were negatively correlated with both inbreeding depression and the waiting time. Species with stronger inbreeding depression exhibited longer waiting times. These patterns obtained on Basommatophorans still hold when including eight other hermaphroditic animals. Our results support the hypothesis that selection drives the evolution of mating-system syndromes in animals. The reaction norm of selfing rates to mate availability is a key target of natural selection in this context.

KEY WORDS: Life-history traits, mollusks, plants, plasticity, reaction norm, reproductive assurance, waiting time.

The evolution of the selfing rate has been interpreted in terms of a diversity of selective forces (Charlesworth and Charlesworth 1979, 1987; Lloyd 1979; Holsinger et al. 1984; Husband and Schemske 1996). Inbreeding depression, the relative reduction in fitness of inbred offspring compared to outbred offspring, is potentially the most important of such forces (Jarne and Charlesworth 1993; Uyenoyama et al. 1993; Barrett and Harder 1996; Husband and Schemske 1996). Lande and Schemske (1985) predicted that inbreeding should decrease the frequency of deleterious, recessive mutations that can reduce the population-level inbreeding depression (i.e., the genetic-purging hypothesis). Accordingly, one expects mating systems to evolve toward one of the two following stable states: low inbreeding depression–high selfing rate or high inbreeding depression–low selfing rate (Lande and Schemske 1985; Charlesworth et al. 1990). Husband and Schemske (1996) provided empirical support for this hypothesis by demonstrating a negative association between selfing rates and inbreeding depression in a metaanalysis of 54 species of vascular plants spanning 23 families. Although there is a general agreement between theory and results, at least at the interspecific level, the observation of a substantial fraction of species with intermediate selfing rates (i.e., mixed mating; Goodwillie et al. 2005; Jarne and Auld 2006) tends to discredit the models predicting either extreme outcrossing or extreme selfing as evolutionary stable states. Other models have identified conditions under which partial selfing can be evolutionarily stable, including pollen discounting (Holsinger 1991), genetic structure (Ronfort and Couvet 1995), and temporal variation in inbreeding depression (Cheptou and Mathias 2001). By examining the pattern of coevolution between the selfing rate and inbreeding depression, we can evaluate the utility of these various models.

Two other sources of dissatisfaction emerge from the current literature on mating-system evolution. First, very little is known about groups other than angiosperms (Jarne et al. 1993; Jarne and Auld 2006; Jordaens et al. 2007; Eppley et al. 2007). The generality of theoretical predictions should be tested in groups that do not share the particulars of flowering plants (e.g., floral biology, coevolution with pollinators, sessile life and a relatively

recent phylogenetic origin). Second, the selfing rate is often considered as a fixed population parameter while it should be better considered as the product of an interaction between trait expression (e.g., anther–stigma separation, gynodioecy, self-incompatibility) and environmental conditions (e.g., pollinator service, population density). It is important here to distinguish two notions: on the one hand, the “actual mating system” in natural populations, which can be predominant selfing, predominant outcrossing, or mixed-mating, depending on the numerical importance of self- and cross-fertilization; on the other hand, the “preferential mating system,” that is, the selfing rates that individuals have when they are free of environmental constraints such as the lack of mates or pollen. Many of the purportedly mixed-mating species may actually be preferential outcrossers submitted to environmental constraints that result in selfing. It is therefore essential to investigate how species respond to environmental constraints, such as mate availability.

In animals (as well as in plants), preferentially outcrossing individuals face a dilemma in the absence of mates (or pollinators): they can either self-fertilize their eggs (or ovules) and pay the cost of selfing (e.g., inbreeding depression), or wait for the arrival of a hypothetical partner (or pollinator) and potentially pay the cost of this delay (e.g., the increased risk of dying before reproduction). Many plant species (Richardson and Stephenson 1989; Dole 1990; Klips and Snow 1997; Kalisz et al. 1999) have evolved a strategy known as delayed selfing (Lloyd 1979) whereby flowers self-fertilize after some time when cross-fertilization has not occurred. Similarly, hermaphroditic animals, including snails (Wethington and Dillon 1997; Tsitrone et al. 2003b) and cestodes (Schjørring 2004) can resort to self-fertilization late in the life cycle when they have not encountered a mating partner. Theory predicts that in preferentially outcrossing animals, isolated individuals should delay their age at first reproduction, compared to individuals with available mates, to wait for future outcrossing (Tsitrone et al. 2003a). Under strong inbreeding depression, individuals should be more reluctant to self-fertilize their eggs, hence exhibiting longer waiting times (Tsitrone et al. 2003a). In preferentially selfing species, individuals are expected to self as soon as they are sexually mature, independent of population density.

In summary, if the selfing rate is under divergent, directional selection and coevolves with inbreeding depression, we expect two opposite trait syndromes to evolve: high inbreeding depression and long waiting time in species with low selfing rates; and low inbreeding depression and no waiting time in species with high selfing rates. Here, we investigate these correlations in hermaphroditic animals. We use a dataset including 25 species for which estimates of at least two of these three parameters are available. Most of the data are from Basommatophoran gastropods, but a few other groups are represented (Stylommatophoran gastropods, bivalves, cestodes, and branchiopods). Part of the dataset was built in the present study (12 species) through a collaboration involving several laboratories. The remaining part was derived from the literature (13 species). Our analysis supports most of the theoretical predictions of directional-selection, inbreeding-depression based models of the evolution of selfing rate, at least in the Basommatophoran group. We discuss the implications of these results for the evolution of mating systems in hermaphroditic animals and compare our results with the current evidence in vascular plants.

Materials and Methods

In animals, hermaphroditism has been reported in 21 phyla (Jarne and Charlesworth 1993; Jarne and Auld 2006) and self-fertilization is known in ascidians, cestodes, cnidarians, mollusks, nematodes, trematodes, and vertebrates (Jarne and Auld 2006). The best studied group with regard to mating systems is arguably Pulmonate gastropods (comprising ~20,000 species; Jarne and Charlesworth 1993; Jarne et al. 1993; Jordaens et al. 2007; Schärer 2009). Studies in other, nongastropod animals capable of self-fertilization are rare, except in some shrimps (Weeks et al. 1999), cestodes (Schjørring 2004; Schjørring and Jäger 2007), and nematodes (Dolgin et al. 2007; Morran et al. 2009a). Because Basommatophoran snails constitute the bulk of our data, we first describe some of their important characteristics before explaining how our datasets were built. This does not mean that we focus on the particulars of Basommatophoran snails; we test the generality of a theory that so far has only been examined and validated in one group (vascular plants) although its main tenets (e.g., the coevolution of inbreeding depression and selfing rate) are not specific to this group. To this aim, we add a second, independent clade. Our results are therefore of concern to all evolutionary biologists interested in mating-system evolution.

BASOMMATOPHORANS: MODELS FOR STUDYING THE EVOLUTION OF MATING SYSTEMS IN ANIMALS

The suborder Basommatophora (Mollusca: Gastropoda) is a monophyletic clade comprising most Pulmonate gastropods liv-

ing in freshwater (Jarne et al. 2010). The group contains far fewer species (~200–300) but is older (~250–300 million years, Myr) than flowering plants (~250,000 species; ~140 Myr). Basommatophorans occupy a diversity of freshwater habitats, including small ditches, ponds, rivers, lakes, and irrigation systems, where marked alternation of high- and low-density episodes are common (Jarne and Städler 1995; Henry et al. 2005; Jarne et al. 2010). Basommatophorans are relatively easy to breed in the laboratory, and most species have fairly short life cycles (~2–10 generations per year in the laboratory). All species are simultaneous hermaphrodites and may reproduce uniparentally through internal self-fertilization and biparentally through cross-fertilization (Jarne et al. 1993; Jarne et al. 2010). Cross-fertilization requires copulation during which one individual acts as male whereas the other acts as female. In general, copulation is not simultaneously reciprocal, although the two partners may switch mating roles sequentially (Koene and Ter Maat 2005). Selfing can be enforced by isolating individuals before sexual maturation (e.g., Larambergue 1939; Paraense and Corrêa 1988; Jarne and Delay 1990; Jarne et al. 1991, 1993, 2000; Städler et al. 1993; Doums et al. 1996b; Njiokou et al. 2000; Henry et al. 2003; Tsitrone et al. 2003b; Escobar et al. 2007; Auld and Relyea 2008). Because outcrossing requires copulation, it cannot be enforced as in plants. The experimenter does not have direct control over copulation rates or on how inseminated individuals use the sperm they receive.

Basommatophorans are oviparous and lay eggs within capsules (clutches) typically containing a few tens of eggs. Hatching occurs typically 1–2 weeks after egg-laying. Each of the three major Basommatophoran families (Lymnaeidae, Physidae, and Planorbidae, comprising ~90% of Basommatophoran species; Jarne et al. 2010) contains known examples of both predominantly outcrossing and predominantly selfing species. In outcrossing species, individuals can store and use foreign sperm for several weeks after copulation (Cain 1956; Wethington and Dillon 1991; Dillon et al. 2005), in such a way that cross-fertilization is effective in the laboratory after the first insemination during long periods without additional pairing. In the predominantly outcrossing species *Biomphalaria glabrata*, *Physa acuta*, and *Lymnaea stagnalis*, all of which have been studied in detail using molecular and morphological markers, most eggs appear to be outcrossed as soon as allosperm is available (Vianey-Liaud et al. 1989; Henry et al. 2005; Koene et al. 2009). In the predominantly selfing species that have been studied so far (*Lymnaea truncatula*, *Bulinus truncatus*, *Bulinus forskalii*, *Biomphalaria pfeifferi*), egg production can take place as soon as individuals are sexually mature, and copulation behavior is rarely observed (Jarne et al. 1993; Doums et al. 1998; Njiokou et al. 2000; Tian-Bi et al. 2008). In these species, cross-fertilization is impossible to enforce.

BASOMMATOPHORAN DATASET

This dataset includes specific estimates of at least two variables out of the following three: selfing rate (S ; derived from molecular markers), apparent inbreeding depression (AID ; see below) and the waiting time (WT) in 17 Basommatophoran species, spanning the Lymnaeidae, Physidae, and Planorbidae. AID and WT of 12 of these species are new experimental data. When available, published data on AID for the same species were added to our dataset. Data on the other five species, as well as all S estimates, were derived from published studies (see references in Table 2). We reanalyzed most of the molecular data used to estimate S using a robust multilocus method (see below).

Details on the origin of individuals of each species are provided in Tables 1 and 2. Except in *B. truncatus*, sampling of the 12 newly studied species was performed between 2004 and 2007 in natural populations from a wide range of countries/continents. Studies in *B. truncatus* were conducted on strains that have been maintained for some time in the laboratory under controlled conditions to estimate WT . Studies were performed in five laboratories in parallel, with separate experiments for each of the 12 species (Table 1). Snails used at the beginning of experiments will be referred to as G_0 individuals.

AID and WT were estimated using a standard protocol, defined by Tsitrone et al. (2003b) and Escobar et al. (2007), as follows. Wild individuals from each species were sampled from one to 12 populations (Table 1) and brought alive to the laboratory where they were placed in 3-l aquariums at 25°C under a 12-h light: 12-h dark photoperiod and fed ad libitum with boiled lettuce twice a week. Water was changed weekly. At the beginning of experiments, G_0 individuals were maintained in high-density conditions (~10 snails per liter) for one week to ensure that individuals had access to partners, thus maximizing copulation opportunities. After this period, G_0 individuals were isolated in 75-mL plastic boxes (Fig. 1). Eggs from G_0 snails were isolated and the offspring (G_1 individuals) were reared under optimal conditions of food and water. Before sexual maturity (~22–30 days depending on the species; Table 1), G_1 snails were split into two treatments: (1) obligate self-fertilization: virgin individuals were isolated throughout their lives, never encountering mates; and (2) facultative cross-fertilization: individuals were isolated most of the time, but encountered adult mates (one partner per period) 3 times a week for 3 h per mating session (i.e., 9 h/week); mates were distinguished by a harmless dot of paint on the shell (Henry and Jarne 2007) and each G_1 individual received a different, randomly chosen partner from the same population and species, maintained in stock colonies, at each mating session. This protocol minimizes density or grouping effects (e.g., competition effects) because individuals spend only 5% of their time as pairs, while allowing snails to copulate frequently as soon as they are sexually mature.

In both treatments, we measured the age at first reproduction (T) of G_1 individuals by checking for the presence of egg capsules every 2–3 days (in some cases every week; Table 1), and estimated the waiting time as $WT = T_S - T_O$, and the relative waiting time as WT/T_O , where T_S and T_O are mean ages at first reproduction in the obligate self-fertilization (s) and the facultative cross-fertilization (o) treatments. In addition, we constructed cumulative histograms of T_S and T_O for each species. These histograms were used to visualize the waiting time directly, as well as to inspect the percentage of individuals that do not reproduce in each treatment (e.g., sterile, self-incompatible or individuals avoiding selfing to all degrees). We also estimated Δ_{\max} , the maximum difference (as a proportion) between T_S and T_O curves (see Fig. 3). Δ_{\max} is expected to be large when WT is large, but it potentially adds information that is not conveyed by WT . First, if some individuals produce absolutely no eggs when isolated, or die before they ever start to lay eggs, they cannot be used in the computation of WT (it becomes infinite) although T_O can be estimated. We consider that if very few isolated individuals ever reproduce while most of paired individuals do, there is strong evidence for selfing avoidance. In practice, such a situation will be characterized by a large Δ_{\max} , although the WT will be very imprecise, being largely influenced by the few individuals that actually selfed. Second, Δ_{\max} is appropriate to compare species with very different life histories as it is independent of the actual time scale.

When T_S , T_O , and WT estimates were derived from more than a single population, the arithmetic mean across populations was calculated to obtain one estimate per species. For Δ_{\max} , we drew the cumulative histograms for all populations and calculated a single estimate. We tested the null hypothesis that WT was zero using t -tests in JMP 3.2.1 (SAS Institute Inc.). To provide a quantitative representation of the effect of the waiting behavior on the selfing rate, we also plotted the expected population selfing rate as a function of population density for various values of the waiting time using the equations derived from Tsitrone et al. (2003a). Δ_{\max} takes values between 0 and 1. To test whether the experimental value exceeded the expectation under the null hypothesis of no effect of treatment on reproduction, we performed 1000 permutations (individuals, including those that never reproduced or died before reproduction, being randomly assigned to treatments) and recalculated Δ_{\max} . The proportion of simulations with Δ_{\max} equal or superior to the observed value was taken as the P -value of our hypothesis test. Permutations were performed with *Mathematica* 4.0 (Wolfram 1996). When necessary, the overall significance in a group of species was obtained by combining P -values across species using Fisher's procedure (Fisher 1925).

Eggs from G_1 individuals from both treatments were counted and isolated in plastic boxes. Hatching typically takes 7–10 days in all species studied under laboratory conditions. Fifteen to 30 days

Table 1. Experimental settings for the new data presented in this study. Pop. = number of populations; Origin = geographical origin of the studied populations; N_{WT} = number of G_1 individuals used to estimate the waiting time; N_{AID} = number of G_2 eggs serving to estimate the apparent inbreeding depression on juvenile survival. Start = starting date of experiments; Isol. = age of isolation before sexual maturity (in days); Checking 1st reproduction refers to how often the isolations were checked for the beginning of reproduction (in days); Survival refers to juvenile survival (0–15 days). All experiments started at laboratory generation 0, except in *B. truncatus* (~40 generations). Guadeloupe and Martinique are Lesser Antilles islands. PA = Pennsylvania; NA = not available.

Family	Species	Pop.	Origin	Coordinates	Laboratory	N_{WT}	N_{AID}	Start	Isol.	Checking 1st reproduction	Survival
Lymnaeidae	<i>Pseudosuccinea columella</i>	1	Colombia	06°07'24"N, 75°27'08"W	Medellin	236	9,541	08/09/04	30	2–3	0–15
	<i>Radix peregra</i>	2	France	43°43'46"N, 03°49'51"E; 43°46'15"N, 03°47'47"E	Montpellier	92	5,400	10/30/06	30	2–3	0–15
Physidae	<i>Physa acuta</i>	12	Guadeloupe (1); France (11)	16°19'16"N, 61°17'58"W; 43°39'55"N, 03°46'45"E; 43°41'08"N, 03°55'05"E; 43°43'46"N, 03°49'51"E; 43°44'42"N, 03°42'30"E; 43°45'19"N, 03°43'37"E; 43°45'35"N, 03°46'38"E; 43°45'56"N, 03°58'37"E; 43°46'10"N, 03°47'29"E; 43°46'15"N, 03°47'47"E; 43°47'46"N, 03°44'33"E; 46°44'41"N, 06°22'52"E	Montpellier	615	96,673	10/21/05	22	2–3	0–15
	<i>Physa gyrina</i>	1	PA (USA)	41°36'20"N, 80°22'56"W	Pittsburgh	100	640	04/11/07	30	2–3	0–15
	<i>Physa marmorata</i>	3	Martinique (1); Guadeloupe (2)	14°48'39"N, 61°02'56"W; 16°16'04"N, 61°27'17"W; 16°21'07"N, 61°27'25"W	Montpellier	245	7,283	02/15/06	22	2–3	0–15
Planorbidae	<i>Biomphalaria glabrata</i>	1	Guadeloupe	16°16'10"N, 61°32'01"W	Perpignan	50	7,492	04/11/05	30	7	NA
	<i>Bulinus truncatus</i>	1	Niger	13°03'41"N, 2°01'48"E	Montpellier	71	6,776	10/19/06	30	2–3	0–15
	<i>Helisoma anceps</i>	1	PA (USA)	41°35'19"N, 80°14'27"W	Pittsburgh	28	434	11/02/07	30	2–3	0–15
	<i>Helisoma duryi</i>	1	Guadeloupe	16°14'49"N, 61°37'05"W	Perpignan	50	NA	09/30/05	30	7	NA
	<i>Helisoma trivolvis</i>	1	PA (USA)	41°35'19"N, 80°14'27"W	Pittsburgh	84	2,484	11/02/07	30	2–3	0–15
	<i>Indoplanorbis exustus</i>	1	Ivory Coast	05°56'27"N, 04°12'12"W	Perpignan	50	71,722	01/20/06	30	7	0–15
	<i>Planorbula armigera</i>	1	PA (USA)	41°36'20"N, 80°22'56"W	Pittsburgh	100	3,492	04/11/07	30	2–3	0–15

Table 2. Parameters of the mating system in Basommatophorans. Values in bold characters correspond to original data contributed by this article. Other data were extracted from the literature (see Notes and Sources). Species are sorted by family and then by selfing rate. *S* = selfing rate; *T* = age at first reproduction (in days); *WT* = waiting time (in days); Δ_{\max} = maximum difference (as a proportion) between *T_S* and *T_O*; *W* = juvenile survival; *AID* = apparent inbreeding depression; *ID_{max}* = maximum inbreeding depression; *o* = outcrossing; *s* = selfing; *ME* = multilocus estimate (David et al. 2007). ****P* < 0.001, ***P* < 0.01, **P* < 0.05; NS = not significant; NA = not available. Note that the null hypothesis tested for *S*, *WT*, and *AID* is that these are zero. The null hypothesis for Δ_{\max} is no effect of the treatment (isolated vs. nonisolated) on reproduction. Marker types are indicated in brackets ([M] = microsatellites; [A] = allozymes).

Species	<i>S</i>	<i>T_O</i>	<i>T_S</i>	<i>WT</i>	Δ_{\max}	<i>W_O</i>	<i>W_S</i>	<i>AID</i>	<i>ID_{max}</i>	Notes and sources
Lymnaeidae										
<i>Lymnaea stagnalis</i>	0.04 ± 0.09 ^{NS(1)}	73.7 ± 6.6 ⁽²⁾	94.5 ± 16.3 ⁽²⁾	20.8 ± 9.8 ^{***(2)}	0.72 ^{***(2)}	NA	NA	0.10 ± 0.11 ^{*(3)}	NA	⁽¹⁾ ME, mean ± SD across eight populations (Puurinen et al. 2007) [M] ⁽²⁾ Mean ± SD across two experiments (Van Duivenboden 1983) ⁽³⁾ On hatching rate, mean ± SD across one (Coutellec and Lagadic 2006) and eight (Puurinen et al. 2007) populations
<i>Radix peregra</i>	0.13 ± 0.17 ^{NS(1)}	90.9 ± 1.8⁽²⁾	103.4 ± 12.6 ± 12.6⁽²⁾	12.6 ± 10.9⁽²⁾	0.37^{*(2)}	0.75 ± 0.04⁽³⁾	0.46 ± 0.13⁽³⁾	0.38 ± 0.20^{*(3)}	0.54⁽³⁾	⁽¹⁾ ME, mean ± SD across three (Jarne and Delay 1990), eight (Coutellec-Vreto et al. 1994), and six populations (M.A. Coutellec, unpubl. data) [A] ⁽²⁾ Mean ± SD across two populations (this study) ⁽³⁾ Mean ± SD across one (Jarne and Delay 1990) and two (this study) populations
<i>Pseudosuccinea columella</i>	0.97 ± 0.02 ^{***(1)}	47.5 ± 4.6⁽²⁾	46.7 ± 3.9⁽²⁾	-0.8 ± 4.2^{NS(2)}	0.01^{NS(2)}	0.79 ± 0.20^{NS(2)}	0.74 ± 0.21⁽²⁾	0.06 ± 0.20^{NS(2)}	0.26⁽²⁾	ME, mean ± SD across three populations (Nicot et al. 2008) [M] ⁽²⁾ Mean ± SD across individuals, one population (this study)
Physidae										
<i>Physa acuta</i>	0.10 ± 0.16 ^{NS(1)}	47.2 ± 5.9⁽²⁾	61.3 ± 10.1⁽²⁾	14.1 ± 7.2^{***(2)}	0.37^{***(2)}	0.53 ± 0.17⁽²⁾	0.25 ± 0.09⁽²⁾	0.54 ± 0.18^{***(2)}	0.75⁽²⁾	⁽¹⁾ ME, mean ± SD across 12 (David et al. 2007) and 10 (Escobar et al. 2008) populations [M+A] ⁽²⁾ Mean ± SD across 12 populations (this study)
<i>Physa gyrina</i>	0.30 ± 0.11 ^{*(1)}	56.0 ± 14.3⁽²⁾	59.2 ± 14.0⁽²⁾	3.2 ± 14.1^{NS(2)}	0.30^{NS(2)}	0.64 ± 0.43 ⁽³⁾	0.50 ± 0.35 ⁽³⁾	0.22 ± 0.02 ^{*(3)}	0.50 ⁽³⁾	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across two populations (Buth and Suloway 1983) [A] ⁽²⁾ Mean ± SD across individuals, one population (this study) ⁽³⁾ Mean ± SD across one (Dillon et al. 2004) and two (McCarthy and Sih 2008) populations
<i>Physa marmorata</i>	1.00 ± 0.002 ^{***(1)}	39.2 ± 1.8⁽²⁾	39.4 ± 5.5⁽²⁾	0.2 ± 4.3^{NS(2)}	0.08^{NS(2)}	0.63 ± 0.37⁽²⁾	0.65 ± 0.33⁽²⁾	-0.10 ± 0.18^{NS(2)}	0.35⁽²⁾	⁽¹⁾ ME, mean ± SD across four populations (Dubois et al. 2008) [M] ⁽²⁾ Mean ± SD across three populations (this study)

Continued.

Table 2. Continued.

Species	S	T _O	T _S	WT	Δ _{max}	W _O	W _S	AID	ID _{max}	Notes and sources
Planorbidae										
<i>Biomphalaria glabrata</i>	0.10 ± 0.18 ⁽¹⁾	49.4 ± 3.5 ⁽²⁾	52.9 ± 5.6 ⁽²⁾	3.5 ± 4.5 ⁽²⁾	0.28 ⁽²⁾	0.70 ± 0.07 ⁽³⁾	0.58 ± 0.01 ⁽³⁾	0.17 ± 0.07 ^{NS(3)}	0.42 ⁽³⁾	⁽¹⁾ ME, mean ± SD across 32 (Mavárez et al. 2002a; Mavárez et al. 2002b) and five (Prugnolle et al. 2005) populations [M] ⁽²⁾ Mean ± SD across individuals, one population (this study) ⁽³⁾ Mean ± SD across one (Costa et al. 2004) and one (this study) populations
<i>Bulinus globosus</i>	0.16 ± 0.26 ^{NS(1)}	NA	NA	NA	NA	0.66 ± 0.10 ⁽²⁾	0.56 ± 0.20 ⁽²⁾	0.16 ± 0.18 ^{NS(2)}	0.44 ⁽²⁾	⁽¹⁾ ME, mean ± SD across six populations (Njiokou et al. 1994) [A] ⁽²⁾ Mean ± SD across one (Jarne et al. 1991) and two (Njiokou et al. 1992) populations
<i>Helisoma anceps</i>	NA	89.6 ± 15.5 ⁽¹⁾	134.0 ± 0.0 ⁽¹⁾	44.4 ± 11.0 ⁽¹⁾	0.86 ⁽¹⁾	0.74 ± 0.35 ⁽¹⁾	0.32 ± 0.19 ⁽¹⁾	0.56 ± 0.28 ⁽¹⁾	0.68 ⁽¹⁾	⁽¹⁾ Mean ± SD across individuals, one population (this study)
<i>Helisoma duryi</i>	NA	50.1 ± 2.6 ⁽¹⁾	110.4 ± 61.4 ⁽¹⁾	60.3 ± 43.5 ⁽¹⁾	0.92 ⁽¹⁾	0.96 ⁽²⁾	0.33 ⁽²⁾	0.66 ⁽²⁾	0.67 ⁽²⁾	⁽¹⁾ Mean ± SD across individuals, one population (this study) ⁽²⁾ Mean of one population (Paraense and Corrêa 1988)
<i>Helisoma trivolvis</i>	NA	104.3 ± 9.7 ⁽¹⁾	137.5 ± 24.0 ⁽¹⁾	33.2 ± 18.3 ⁽¹⁾	0.79 ⁽¹⁾	0.84 ± 0.20 ⁽¹⁾	0.00 ⁽¹⁾	1.00 ⁽¹⁾	1.00 ⁽¹⁾	⁽¹⁾ Mean ± SD across individuals, one population (this study)
<i>Planorbula armigera</i>	NA	74.2 ± 7.1 ⁽¹⁾	137.1 ± 8.1 ⁽¹⁾	62.9 ± 7.6 ⁽¹⁾	1.00 ⁽¹⁾	0.72 ± 0.14 ⁽¹⁾	0.29 ± 0.46 ⁽¹⁾	0.60 ± 0.34 ⁽¹⁾	0.71 ⁽¹⁾	⁽¹⁾ Mean ± SD across individuals, one population (this study)
<i>Bulinus truncatus</i>	0.83 ± 0.02 ⁽¹⁾	38.0 ± 4.2 ⁽²⁾	36.4 ± 4.2 ⁽²⁾	-1.6 ^{NS(2)}	0.08 ⁽²⁾	0.83 ± 0.15 ⁽³⁾	0.85 ± 0.15 ⁽³⁾	-0.02 ± 0.02 ^{NS(3)}	0.15 ⁽²⁾	⁽¹⁾ ME, mean ± SD across three populations (Viard et al. 1997) [M] ⁽²⁾ Mean ± SD across individuals, one population (this study) ⁽³⁾ Mean ± SD across two (Doums et al. 1996a) and one (this study) populations
<i>Biomphalaria kuhniana</i>	0.84 ± 0.13 ⁽¹⁾	NA	NA	NA	NA	0.76 ± 0.18 ⁽²⁾	0.78 ± 0.11 ⁽²⁾	-0.04 ± 0.11 ^{NA(2)}	0.22 ⁽²⁾	⁽¹⁾ ME, mean ± SD across two populations (Dupuy et al. 2009) [M] ⁽²⁾ Mean ± SD across one (Doums et al. 1996b) and one (Costa et al. 2004) populations
<i>Biomphalaria pfeifferi</i>	0.94 ± 0.15 ⁽¹⁾	101.4 ± 8.2 ⁽²⁾	96.3 ± 11.0 ⁽²⁾	-5.2 ± 2.8 ⁽²⁾	0.14 ^{NS(2)}	0.62 ± 0.08 ⁽²⁾	0.77 ± 0.02 ⁽²⁾	-0.25 ± 0.19 ⁽²⁾	0.23 ⁽²⁾	⁽¹⁾ Derived from F _{is} , mean ± SD across 24 (Charbonnel et al. 2002a) and 20 (Charbonnel et al. 2002b) populations [M] ⁽²⁾ Mean ± SD across two populations (Tian-Bi et al. 2008)
<i>Biomphalaria tenagophila</i>	NA	69.0 ⁽¹⁾	69.0 ⁽¹⁾	0.0 ^{NA(1)}	NA ⁽¹⁾	0.48 ⁽¹⁾	0.49 ⁽¹⁾	-0.01 ^{NA}	0.51 ⁽¹⁾	⁽¹⁾ Mean of one population (Tuan and Simões 1998)
<i>Indoplanorbis exustus</i>	NA	42.8 ± 4.5 ⁽¹⁾	44.5 ± 3.8 ⁽¹⁾	1.7 ± 4.2 ^{NS(1)}	0.20 ^{NS(1)}	0.73 ± 0.22 ⁽¹⁾	0.73 ± 0.24 ⁽¹⁾	0.01 ± 0.23 ^{NS(1)}	0.27 ⁽¹⁾	⁽¹⁾ Mean ± SD across individuals, one population (this study)

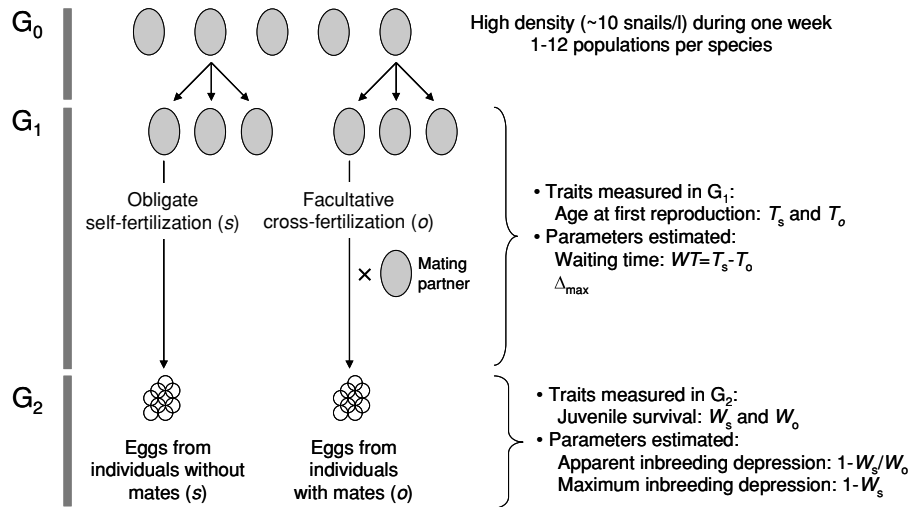


Figure 1. Schematic diagram of the experimental protocol used to estimate the waiting time and apparent inbreeding depression in Basommatophorans. Filled ovals refer to individuals. G_i refers to generations. More details are provided in the text.

after egg-laying, live (free-moving) individuals were counted. Juvenile survival (W) of G_2 snails was calculated in each treatment as the number of living hatchlings divided by the number of eggs laid. Subsequently, the “apparent inbreeding depression” on juvenile survival was estimated as $AID = 1 - W_s/W_o$, where W_s and W_o stand for the mean juvenile survival of offspring from the obligate self-fertilization and the facultative cross-fertilization treatments, respectively. Note that we refer to apparent inbreeding depression rather than to inbreeding depression because outcrossing cannot be enforced in selfing species (i.e., contrary to hand-pollination in plants). In outcrossing species, in which cross-fertilization readily occurs after copulation, this provides an estimate of the actual inbreeding depression (Jarne et al. 1991). However, in selfing species, it provides only the fitness ratio of offspring from individuals with and without mates. Nevertheless, because survival cannot exceed 1, the parameter $1 - W_s$ provides an upward limit to inbreeding depression in selfing species (Jarne et al. 1992); it will be referred to as maximum inbreeding depression (ID_{max}). Note that we focused on juvenile survival because inbreeding depression is usually largest at this stage of the life cycle (Jarne et al. 2000; Escobar et al. 2008) and because the largest differences in inbreeding depression among selfing and outcrossing plant species occur in early components of fitness (Husband and Schemske 1996). As above, when estimates were derived for more than one population, mean W_s , W_o , AID , and ID_{max} were calculated per species. In this case, we tested the null hypothesis that average AID was zero using t -tests.

In addition to these experimental data, estimates of WT , Δ_{max} , AID , and ID_{max} , or their basic components (i.e., T and W), were obtained in five species from literature searches (ISI Web of Science). We selected studies with protocols comparable to ours, including both isolated and nonisolated snails.

We also obtained molecular estimates of the selfing rate (S) in 11 species. The fixation index F_{is} has been extensively used to estimate selfing rates using $S = 2F_{is}/(1 + F_{is})$, which assumes inbreeding equilibrium. However, important upward biases are associated with this method, especially in outcrossing species, because of null alleles and mis-scoring (David et al. 2007; Jarne and David 2008). When possible (five species), we estimated S using the original datasets and a more robust multilocus method based on maximum likelihood, which has been shown to be insensitive to mis-scoring and does not depend on F_{is} (RMES software, available at <ftp://ftp.cefe.cnrs.fr/>; David et al. 2007). When these datasets were not available, S estimates were derived from F_{is} . The null hypothesis $S = 0$ was tested per population either using RMES or, when unavailable, from the significance of F_{is} values provided in the published papers. When several population estimates were available per species, mean selfing rates were calculated across populations, and the overall significance was obtained by combining population P -values using Fisher’s procedure. Selfing rates could be estimated in 11 of 17 Basommatophoran species. Note that this dataset does not include all available selfing rates in Basommatophorans. We only included species in which at least one estimate of AID and/or WT was available. Note also that selfing rates were sometimes estimated from populations different from those used in our experiments (and sometimes much more numerous). However, except for *B. pfeifferi* and *B. straminea*, the S estimates included populations geographically close to ours. Moreover, the observed variance of selfing rates among populations within species was on average very low (0.02), 8.5 times lower than the variance among species (0.17) so the choice of populations cannot greatly affect interspecific patterns. Average selfing rates were used to classify species as outcrossers ($S < 0.2$), selfers ($S > 0.8$), or intermediate.

NON-BASOMMATOPHORAN DATASET

We retrieved eight non-Basommatophoran hermaphroditic species for which at least two of three parameters (*AID*, *WT*, and *S*), or their component terms, could be estimated. Data were analyzed as described above. The non-Basommatophoran dataset is very heterogeneous in taxonomical origin: it includes five terrestrial Pulmonate gastropods (Stylommatophora), one bivalve, one cestode, and one branchiopod (Table 3). We do not pretend to provide a comprehensive view of hermaphroditic animals with these few species, rather we want to explore whether the few data that can be found are in agreement with the patterns observed in Basommatophorans.

CORRELATION ANALYSES

As in previous large-scale studies (Husband and Schemske 1996; Goodwillie et al. 2005; Jarne and Auld 2006) our dataset is made of a single average estimate of each variable per species. Non-parametric correlations (Spearman's rho) were computed between parameter pairs and the α -level was adjusted for multiple comparisons (Verhoeven et al. 2005). Correlations were calculated on both the Basommatophoran and complete datasets using JMP 3.2.1 (SAS Institute Inc.). For the graphical representation, the waiting time was transformed as $\ln(11+WT)$ to homogenize variances and decrease the influence of outliers (the constant 11 was chosen to obtain close to normal distributions and avoid infinite values; transformation does not affect nonparametric tests). For the Basommatophoran dataset, correlations were also estimated using phylogenetic independent contrasts (PICs) with Mesquite 2.72 (Maddison and Maddison 2009). No comprehensive Basommatophoran phylogeny is available. A phylogeny including most Basommatophoran species studied here was built using the mitochondrial cytochrome oxidase I (*COI*) gene (sequences available in GenBank). *Helix aspersa* (Pulmonata: Stylommatophora) was used as outgroup. Alignments were obtained with Clustal-W and the phylogeny was built using a GTR + G + I model with PhyML 3.0 (Guindon and Gascuel 2003) (Fig. S1).

As stated in the introduction, the theoretical model (Tsitrone et al. 2003a) predicts that a positive waiting time should evolve only in preferential outcrossers with high inbreeding depression. Correlations including all species should therefore emerge from the contrast between two clusters of species: preferential selfers with no waiting time and low *AID* versus preferential outcrossers with positive waiting time and high *AID*. However the model also predicts that, within preferential outcrossers, the waiting time should quantitatively increase with inbreeding depression. We tested this hypothesis by redoing the correlation tests, keeping only species with positive and significant *WT* and/or Δ_{\max} (i.e., preferential outcrossers, based on their reproductive behavior).

Results

BASOMMATOPHORAN DATASET

Five Basommatophoran species exhibited $S < 0.2$, most of which are not significantly different from zero (hereafter, outcrossing species). Five other species had selfing rates higher than 0.8 (hereafter, selfing species), all of which were significantly different from zero (Table 2; Fig. 2A). Selfing and outcrossing species were detected within each of the three studied families of Basommatophorans. One species (*Physa gyrina*) seems to classify as a mixed-mater ($S = 0.3$ based on F_{is} at three weakly polymorphic allozyme loci typed in 54 individuals from two populations; Buth and Suloway 1983). Unfortunately, the original dataset of Buth and Suloway (1983) was not available for re-analysis using multi-locus methods. We will group *P. gyrina* with outcrossing species for further comparisons between selfing and outcrossing species. Excluding it does not alter our conclusions.

We also obtained new data on *WT*, Δ_{\max} , *AID* and ID_{\max} in 11 Basommatophoran species. Data were already available in several other species (see Table 2 for references). All outcrossing species (*L. stagnalis*, *R. peregra*, *P. acuta*, *P. gyrina* and *B. glabrata*) had positive *WT* (mean \pm SD across species; 10.8 ± 7.5 days, $t_4 = 3.23$, $P = 0.03$); values significantly differed from 0, except in *P. gyrina*. Mean Δ_{\max} for outcrossing species was 0.41 ± 0.18 ($P < 0.0001$). In selfing species (*Pseudosuccinea columella*, *Physa marmorata*, *B. pfeifferi*, *B. truncatus*) *WT* did not differ from zero on average (-3.2 ± 4.9 days, $t_3 = 1.29$, $P = 0.29$), except in *B. pfeifferi* where it was negative (-5.2 days, $P < 0.05$) meaning that isolated individuals reproduced before paired individuals. Mean Δ_{\max} in selfing species was 0.08 ± 0.05 ($P = 0.77$). *WT* in four species with no selfing-rate estimates (*Helisoma anceps*, *H. duryi*, *H. trivolvis*, and *Planorbula armigera*) were quite long (33–63 days, $t_3 = 7.18$, $P = 0.006$), representing increases of 32–120% in the age at first reproduction (Fig. S2A). Consistently, Δ_{\max} for *Helisoma* spp. and *P. armigera* were high (0.79–1.00, $P < 0.0001$; Fig. 3). In contrast, *B. tenagophila* and *Indoplanorbis exustus* exhibited nonsignificant waiting times of 0.0 and 1.7 days (or 0.0% and 3.9%) respectively. Δ_{\max} in *I. exustus* is 0.20 ($P = 0.14$; Fig. 3) but could not be estimated in *B. tenagophila*.

Apparent inbreeding depression was estimated in 17 species. Considering the 11 species for which both *S* and *AID* are known, the average *AID* for outcrossing species was 0.26 ± 0.17 ($t_5 = 3.86$, $P = 0.01$) compared to -0.12 ± 0.22 for selfing species ($t_4 = 1.22$, $P = 0.29$) (Fig. S2B). ID_{\max} was significantly higher for outcrossers than selfers (0.53 ± 0.13 and 0.24 ± 0.07 , respectively; $t_8 = 4.28$, $P = 0.003$). *Helisoma* spp. and *P. armigera* (unknown *S* and long *WT*) exhibited high *AID* (0.56–1.00, $P = 0.006$), including cases in which no viable eggs were obtained from isolated individuals. *Biomphalaria tenagophila* and *I. exustus* (unknown *S* and nonsignificant, low *WT*) exhibited low and nonsignificant *AID*

Table 3. Parameters of the mating system in non-Basommatophorans. Species are sorted by taxonomic group and then by selfing rate. *S* = selfing rate; *T* = age at first reproduction; *WT* = waiting time; *W* = juvenile survival; *AID* = apparent inbreeding depression; *ID_{max}* = maximum inbreeding depression; *o* = outcrossing; *s* = selfing. ****P* < 0.001, ***P* < 0.01, **P* < 0.05; NS = not significant; NA = not available. Hypothesis tests are as in Table 2. Marker types are indicated in brackets ([M] = microsatellites; [A] = allozymes).

Species	<i>S</i>	<i>T₀</i>	<i>T_s</i>	<i>WT</i>	<i>W₀</i>	<i>W_s</i>	<i>AID</i>	<i>ID_{max}</i>	Notes and sources
<i>Bivalvia</i>									
<i>Argopecten irradians</i>	0.29 ± 0.07*** ⁽¹⁾	NA	NA	NA	NA	NA	0.40 ^{NA(2)}	NA	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across three populations (Wang et al. 2007) [A] ⁽²⁾ Mean of Stock B (Zhang et al. 2007)
<i>Branchiopoda</i>									
<i>Eulimnadia texana</i>	0.69 ± 0.14 ^{NA(1)}	NA	NA	NA	NA	NA	0.22 ± 0.02* ⁽²⁾	NA	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across four (Weeks et al. 1999) and seven (Weeks and Zucker 1999) populations [A] ⁽²⁾ Mean ± SD across four populations (Weeks et al. 1999)
<i>Cestoda</i>									
<i>Schistocephalus solidus</i>	0.10 ^{NA(1)}	10.5 ⁽²⁾	20.5 ⁽²⁾	10.0*** ⁽²⁾	0.29 ± 0.09 ⁽³⁾	0.06 ± 0.04 ⁽³⁾	0.78 ± 0.08** ⁽³⁾	0.93 ⁽³⁾	⁽¹⁾ Derived from <i>F_{is}</i> , mean of one population (Lüscher and Milinski 2003) [M] ⁽²⁾ Mean of one population (Schjørring 2004). ⁽³⁾ Mean ± SD across three studies (Schjørring 2004; Milinski 2006; Schjørring and Jäger 2007)
<i>Stylommatophora</i>									
<i>Arianta arbustorum</i>	0.50 ± 0.28*** ⁽¹⁾	NA	NA	NA	0.48 ⁽²⁾	0.30 ⁽²⁾	0.37* ⁽²⁾	0.70 ⁽²⁾	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across two populations (Armbruster et al. 2005) [A] ⁽²⁾ Mean of one population (Chen 1993)
<i>Succinea putris</i>	0.51 ± 0.08*** ⁽¹⁾	NA	NA	NA	0.79 ⁽²⁾	0.02 ⁽²⁾	0.97 ^{NA(2)}	0.98 ⁽²⁾	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across four populations (reference plot; Jordaens et al. 2006) [A] ⁽²⁾ Mean of one population (Dillen et al. 2009)
<i>Balea perversa</i>	0.90 ± 0.12 ^{NA(1)}	NA	NA	NA	0.98 ± 0.03 ⁽²⁾	0.96 ± 0.06 ⁽²⁾	0.02 ± 0.05 ^{NS(2)}	0.04 ⁽²⁾	⁽¹⁾ Derived from <i>F_{is}</i> , mean ± SD across treatments (Wirth et al. 1997) [A] ⁽²⁾ Mean ± SD across individuals, one population (Baur and Baur 2000)
<i>Partula taeniata</i>	NA	145.0 ⁽¹⁾	750.0 ⁽¹⁾	605.0*** ⁽¹⁾	549 ⁽²⁾	11 ⁽²⁾	0.98*** ⁽³⁾	NA	⁽¹⁾ Mean of six localities (Murray and Clarke 1966) [A] ⁽²⁾ Number of hatchlings (Murray and Clarke 1966) ⁽³⁾ On the number of hatchlings (Murray and Clarke 1966)
<i>Triodopsis albolabris</i>	NA	65.4 ⁽¹⁾	166.7 ⁽¹⁾	101.3* ⁽¹⁾	0.73 ⁽¹⁾	0.03 ⁽¹⁾	0.96 ^{NA(1)}	0.97 ⁽¹⁾	⁽¹⁾ Mean of two populations (McCracken and Brussaard 1980) [A]

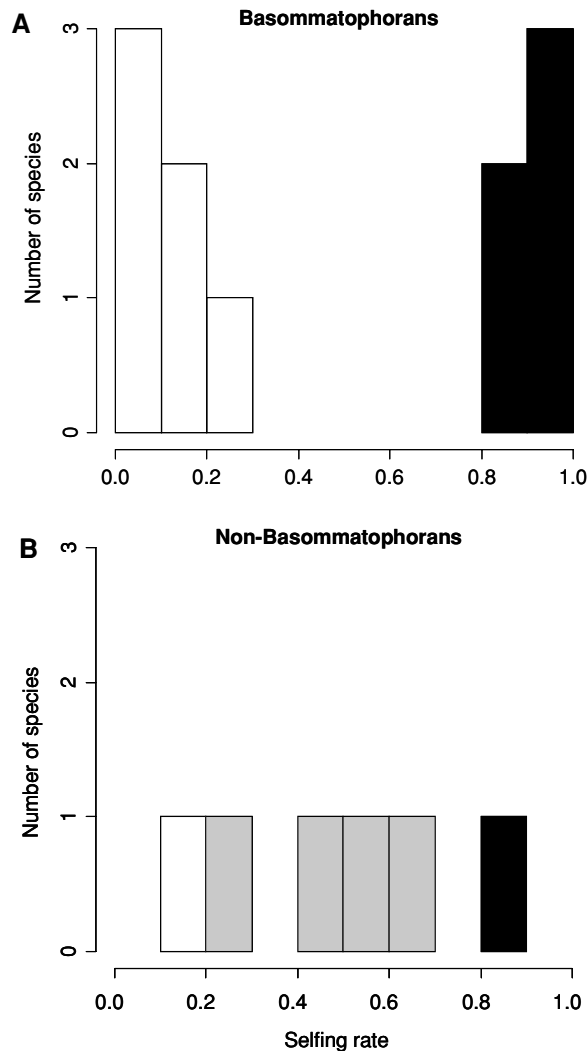


Figure 2. Distribution of selfing rate among Basommatophorans (A) and non-Basommatophorans (B). Black bars = selfing species; open bars = outcrossing species; gray bars = mixed-mating species.

(-0.01 and 0.01 , respectively) (Fig. S2B). Consistently, the three *Helisoma* species and *P. armigera* exhibited strong ID_{\max} (0.68 – 1.00), whereas *I. exustus* and *B. tenagophila* exhibited lower values (0.27 and 0.51 , respectively) (Table 2).

NON-BASOMMATOPHORAN DATASET

This dataset comprises only eight species. Δ_{\max} could not be estimated in any of these species because data on individual reproduction were not available. Selfing rates were estimated in six species, all using the F_{is} method; one of these species exhibited $S < 0.2$, four had $0.2 < S < 0.8$, and one had $S > 0.8$ (Fig. 2B). The WT was available in three species: the cestode *Schistocephalus solidus* (10 days), and the land snails *Partula taeniata* (605 days) and *Triodopsis albolabris* (101 days) (Fig. S2C). These waiting

times represent between 95% and 417% of increase in the age at first reproduction when mates were absent. Of the eight species making up this dataset, four exhibited strong AID (0.78 – 0.98 ; *S. solidus*, *T. albolabris*, *Succinea putris*, and *P. taeniata*), three exhibited moderate values (0.22 – 0.40 ; *Eulimnadia texana*, *Arianta arbustorum*, and *Argopecten irradians*) and one species had low AID (0.02 ; *Balea perversa*) (Fig. S2D). Although it could not be estimated in all non-Basommatophoran species, ID_{\max} shows the same trend as the AID (Table 3).

CORRELATIONS AMONG THE SELFING RATE, APPARENT INBREEDING DEPRESSION, AND THE WAITING TIME

Three major, significant correlations were found. We observed a negative correlation between S and AID (Basommatophoran dataset: $\rho = -0.73$, $P = 0.01$; with PICs: $r_{PIC} = -0.76$, $P = 0.006$; complete dataset: $\rho = -0.64$, $P = 0.006$; Fig. 4A), a negative correlation between S and WT (Basommatophoran dataset: $\rho = -0.77$, $P = 0.005$; $r_{PIC} = -0.81$, $P = 0.008$; complete dataset: $\rho = -0.75$, $P = 0.0005$; Fig. 4B), and a positive correlation between AID and WT (Basommatophoran dataset: $\rho = 0.86$, $P < 0.0001$; $r_{PIC} = 0.90$, $P < 0.0001$; complete dataset: $\rho = 0.69$, $P = 0.0001$; Fig. 4C). These correlations persist when using relative WT , Δ_{\max} instead of WT , and ID_{\max} instead of AID (Table 4). We also found that WT was positively correlated with T_S but not with T_O , and that AID is negatively correlated with W_S but not with W_O . In consequence, AID was positively correlated with T_S but not with T_O in both datasets (Table 4). Finally, positive correlations between T_S and T_O were observed in both datasets.

Plotting W_S against W_O and T_S against T_O , provides a simple visualization of mating system syndromes (Fig. 5), and allows one to position species that do not have selfing-rate estimates (*B. tenagophila*, *Helisoma* spp., *P. armigera*, and *I. exustus*). Overall, *B. tenagophila* and *I. exustus* behave as selfers (the points are located on the lines $T_O = T_S$ and $W_O = W_S$), whereas the three *Helisoma* species and *P. armigera* behave as outcrossers, exhibiting even larger T_S and lower W_S values than all known outcrossers (Fig. 5). Outside Basommatophorans, the same syndrome is found in *T. albolabris*, *P. taeniata*, and *S. solidus*, all of which have high WT and AID values.

When restricting the dataset to preferential outcrossers (species with significantly positive waiting time or Δ_{\max} , including 10 snails plus the cestode *S. solidus*), we still observe positive correlations between AID and estimates of waiting time and Δ_{\max} (Spearman ρ : relative WT (in %) vs. AID : $\rho = 0.79$, $P = 0.004$; absolute WT (in days) vs. AID : $\rho = 0.56$, $P = 0.07$; Δ_{\max} vs. AID : $\rho = 0.71$, $P = 0.03$).

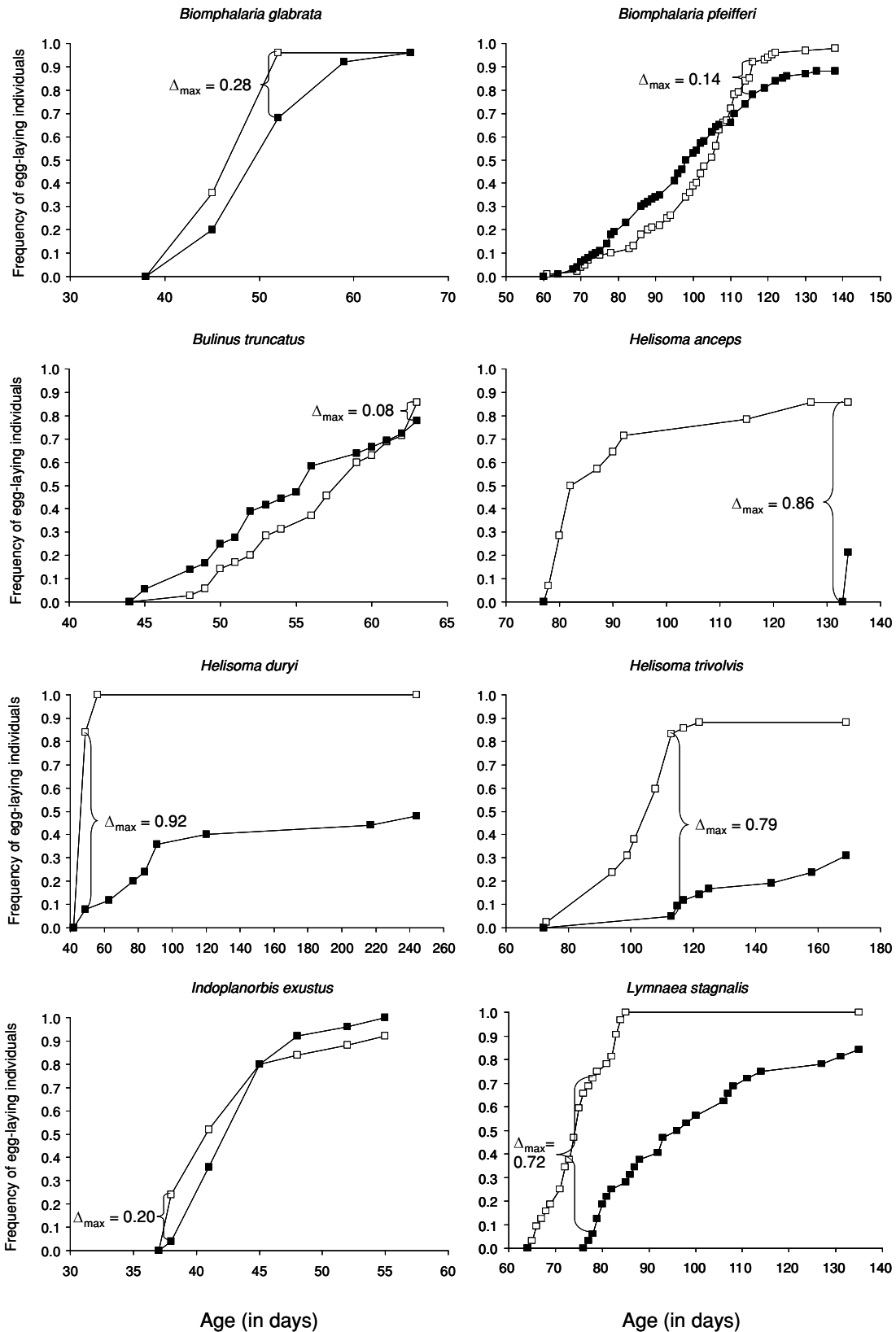


Figure 3. Cumulative proportion of reproducing individuals as a function of age (in days). Open squares: individuals from the facultative cross-fertilization treatment; filled squares: individuals from the obligate self-fertilization treatment. Δ_{max} can be derived from these curves (see top left panel). Each panel represents a species. The 12 species for which new data are reported are represented, as well as *Biomphalaria pfeifferi* and *Lymnaea stagnalis* (data from Tian-Bi et al. 2008 and Van Duivenboden 1983, respectively).

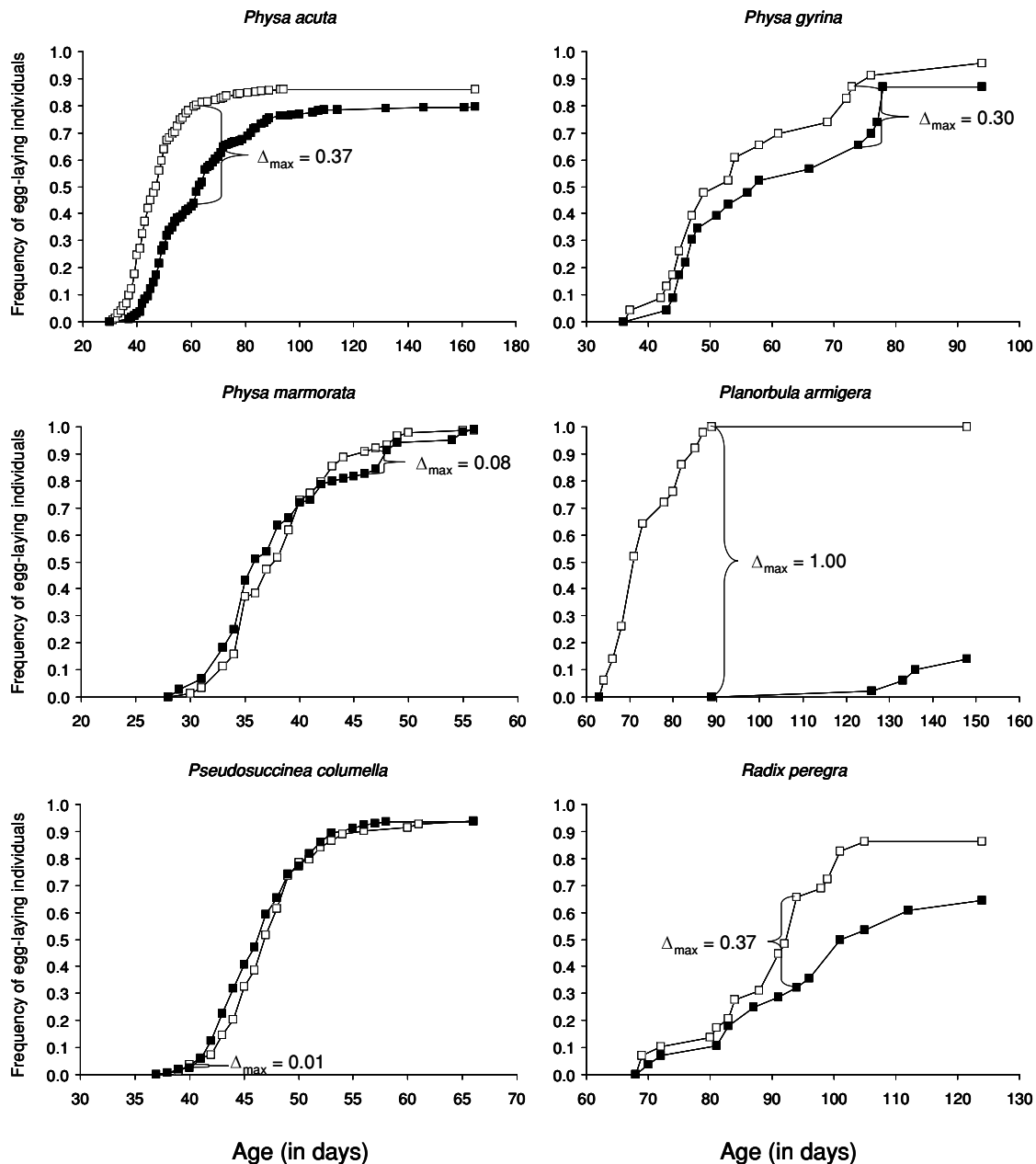


Figure 3. Continued.

Discussion

SELFING RATE IN HERMAPHRODITIC ANIMALS

We observed a strongly bimodal distribution of the selfing rate in Basommatophorans. Such a distribution is consistent with theoretical models predicting directional selection toward high or low selfing rates, and evolutionary instability of mixed-mating systems (Lande and Schemske 1985; Schemske and Lande 1985; Goodwillie et al. 2005). When including data from non-Basommatophoran species, the distribution seems much less bimodal and reminiscent of the relatively flat shape previously observed in animals (Jarne and Auld 2006). However, the dif-

ference between our Basommatophoran dataset and other animal datasets might bear on data quality and methods of estimation rather than on any biological differences. Most of the selfing rates are estimated with the multilocus maximum-likelihood method in Basommatophorans, while they are estimated using F_{is} in other species. Molecular estimates of selfing rates are not without bias (Jarne and David 2008). One well-known bias, common to the two methods, is that inbreeding depression may occur before sampling, and therefore estimates are not “primary” selfing rates. Another bias is specific to the F_{is} method, and arises because null alleles, as well as many other kinds of scoring errors, are

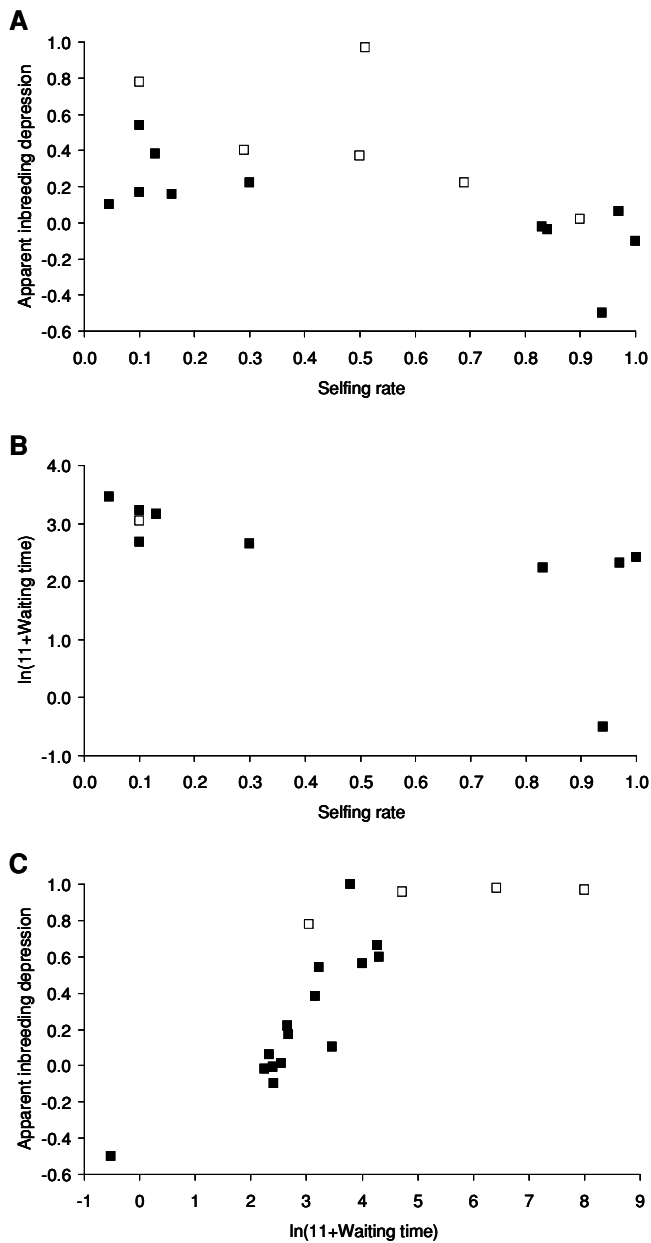


Figure 4. Correlations among selfing rate, apparent inbreeding depression, and the waiting time. Filled squares = Basommatophorans; open squares = non-Basommatophorans. Each point represents a species.

unavoidable and systematically tend to create heterozygote deficiencies resulting in inflated selfing-rate estimates. Using the species in which both F_{is} - and ML-based methods are available (see Table S1) it seems clear that, although there is not much difference between the two methods for highly selfing species, a good proportion (3/5) of the species classified as outcrossers by the ML method would be classified as mixed-maters using F_{is} (see David et al. 2007 for other examples). The fact that all the mixed-maters found in our dataset are species in which we could not access the raw data and had to use F_{is} -based estimates of S raises suspicion

on the reality of mixed mating in animals. In addition, some of these data have other problems. In *P. gyrina*, the F_{is} is actually based on very little information: the product of the sample size by number of loci by average genetic diversity is 8.42 (Buth and Suloway 1983), meaning that heterozygote deficiency is computed on the basis of eight expected observations in total. In *S. putris*, the authors (Jordaens et al. 2006) found a large variance in F_{is} among loci and concluded that the F_{is} observed are inflated by other causes than selfing, which is consistent with the fact that selfed offspring practically never survive (AID is 0.97).

Jarne and Auld (2006) previously highlighted that the global distribution of selfing rates among hermaphroditic animals looks less U-shaped than in plants (Goodwillie et al. 2005). Given that most selfing rates in animals were derived from F_{is} , it is crucial to know what proportion of mixed maters are simply inaccurate estimations. In the light of the present study, it seems (1) that bimodality is strong in Basommatophorans; (2) that predominant selfing and predominant outcrossing have evolved several times in Pulmonate gastropods because we find the two systems in each of the three Basommatophoran families studied, and even within genera (*Biomphalaria*, *Bulinus*, and *Physa*), as well as in the Stylommatophora (terrestrial snails); (3) that the bias introduced by technical artifacts on previous results should be taken into account to identify robust examples of mixed mating. Even if they end up being exceptions to the general rule, these examples are worth detailed investigation. For example, *E. texana* seems to combine mixed-mating and substantial inbreeding depression (although the juvenile AID reported in our table is 0.22, cumulative inbreeding depression estimates over a lifetime range from 0.50 to 0.68; Weeks et al. 2000); this species is unique in our dataset by its androdioecious sexual system (co-occurrence of males and hermaphrodites) whose stability requires specific conditions (Pannell 2008; Chasnov 2010).

SELFING RATE AND INBREEDING DEPRESSION

We expected AID to be higher in outcrossing than in selfing species for two reasons. First, even when mates are available and copulations occur, a large fraction of eggs may be systematically self-fertilized in preferentially selfing species; therefore, only a (potentially small) fraction of offspring from the facultative outcrossing treatment may actually be products of outcrossing, reducing the observed AID . Second, inbreeding depression is predicted to be low in selfers both because recessive, deleterious alleles are more often exposed to selection, especially semi-lethals expressed at early stages (Lande and Schemske 1985; Husband and Schemske 1996) and because selfing is expected to evolve when inbreeding depression is low. The prediction of higher AID in predominant outcrossers is fulfilled in both the Basommatophoran and complete datasets presented here. None of the species classified as selfers had a significantly positive AID , while most

Table 4. Correlation coefficients between parameters of the mating system. For Basommatophorans, raw correlations (Spearman's rho) and phylogenetic independent contrasts (PICs r) are given above the diagonal (PICs are given within parentheses). For the complete dataset, rho is given below the diagonal. T = age at first reproduction; WT = waiting time; W = juvenile survival; AID = apparent inbreeding depression; ID_{\max} = maximum inbreeding depression; S = selfing rate; o = outcrossing; s = selfing. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; NA = not reported because Δ_{\max} could be estimated only in basommatophorans. Correlations in bold characters are still significant after correction for multiple comparisons.

	S	T_o	T_s	WT	Δ_{\max}	W_o	W_s	AID	ID_{\max}
S		-0.28 (0.31)	-0.47 (0.07)	-0.77** (-0.81**)	-0.86** (-0.79*)	0.14 (0.16)	0.65 (0.90)	-0.73* (-0.76**)	-0.65* (-0.90***)
T_o	-0.07		0.86*** (0.73**)	0.36 (-0.40)	0.56* (0.18)	0.05 (-0.09)	-0.47 (-0.16)	0.41 (-0.15)	0.47 (0.16)
T_s	-0.21	0.86***		0.72** (0.23**)	0.86*** (0.77**)	0.20 (0.17)	-0.78** (-0.60*)	0.72** (0.40)	0.78** (0.60*)
WT	-0.75***	0.44	0.76***		0.96*** (0.76**)	0.21 (0.31)	-0.85*** (-0.76**)	0.86*** (0.90***)	0.85*** (0.76**)
Δ_{\max}	NA	NA	NA	NA		0.20 (0.20)	-0.85*** (-0.86**)	0.86*** (0.73*)	0.85*** (0.86**)
W_o	0.39	0.22	0.33	0.04	NA		0.03 (0.06)	0.37 (0.34)	-0.03 (-0.06)
W_s	0.64*	-0.28	-0.58*	-0.66**	NA	0.18		-0.87*** (-0.87***)	-1.00*** (-1.00***)
AID	-0.64**	0.34	0.62**	0.69***	NA	0.16	-0.90***		0.87*** (0.87***)
ID_{\max}	-0.64*	0.28	0.58*	0.66**	NA	-0.18	-1.00***	0.90***	

of the outcrossers had high and significant AID. AID was quite variable among outcrossing species (0.10–1.00 including the three *Helisoma* species, which appear to be outcrossers). One source of this variation is probably the fact that juvenile survival is not measured exactly in the same way in different species; despite our efforts, juveniles were not always checked at the exact same age and a given age (in days) may have different meanings in species with short versus long life cycles. However, we believe that two qualitatively different patterns can be observed, which are not due to technical artifacts. Although some species produce nearly no living juveniles when forced to self-fertilize (AID close to 1.0; e.g., *Helisoma* spp., *P. taeniata*, *S. putris*), others are still able to successfully self-fertilize, although they do suffer from inbreeding depression (e.g., *P. acuta*, *L. stagnalis*). This suggests that the “outcrosser” category includes species that are nearly incapable of efficient reproduction by self-fertilization (as previously observed, for instance, in ascidians; Grosberg 1988), whereas other species can resort to self-fertilization to persist in conditions of low density or otherwise unavailable mates. This situation is reminiscent of angiosperms in which predominantly outcrossing species contain both plants that can self-fertilize in the absence of pollinators (or when inflorescences are artificially bagged) and self-incompatible plants unable to set viable seed autonomously.

Basic models of the evolution of selfing predict that outcrossing is stable if inbreeding depression exceeds a threshold value of 0.5, because genes are transmitted to offspring twice as efficiently through selfed than through outcrossed eggs or seeds. The threshold can decrease below 0.5 if selfing conveys some costs (e.g., pollen/sperm discounting; Lande and Schemske 1985; Charlesworth et al. 1990; Holsinger 1991). Five of 10 purportedly outcrossing species had $AID > 0.5$. The remaining five species, including a relatively modest value (0.10 in *L. stagnalis*), do not directly contradict theoretical predictions, however, because AID was measured only at the very beginning of the life-cycle (0–1 week after hatching). Although hatching and early survival often represent a substantial fraction of the variance in fitness, AID represents only a lower bound for total inbreeding depression. The latter might be greater than 0.5 in some of these species.

AID observed in predominantly outcrossing species is probably due to actual inbreeding depression (i.e., the expression of deleterious recessive mutations). However, the low AID observed in self-fertilizing species can reflect either low inbreeding depression or low propensity of paired individuals to effectively outcross their eggs, as both are expected in predominant selfers. Genetic markers would be required to quantify their respective contribution to AID (see e.g., Weeks et al. 2000). Yet, even restricting to the self-fertilization treatment (for which there is no

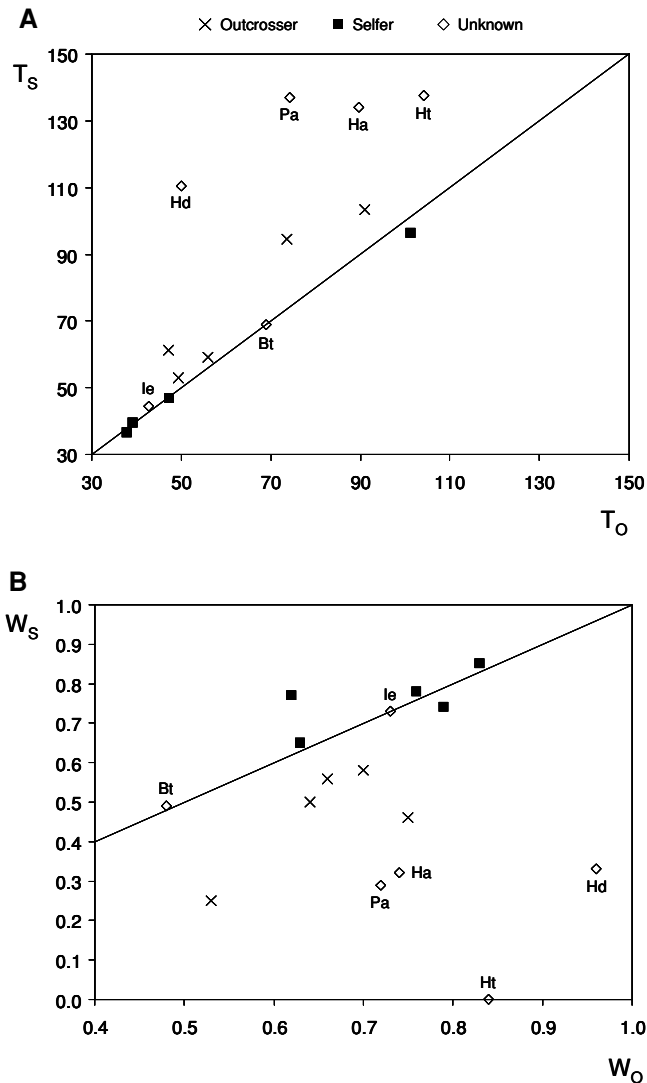


Figure 5. (A) Age at first reproduction of isolated individuals (T_S) as a function of individuals having access to mates (T_O). (B) As in (A) for juvenile survival (W). Lines represent expectations under equality between the two treatments ($T_S = T_O$ and $W_S = W_O$). Each point represents a Basommatophoran species. Bt = *Biomphalaria tenagophila*; Ha = *Helisoma anceps*; Hd = *Helisoma duryi*; Ht = *Helisoma trivolvis*; le = *Indoplanorbis exustus*; Pa = *Planorbula armigera*.

ambiguity in inbreeding level of the offspring relative to their parents), selfing and outcrossing species clearly differ, with larger juvenile survival (W_S) in the former. W_S defines an upper bound for inbreeding depression; thus, the maximum inbreeding depression (assuming 100% survival of outcrossed offspring) of outcrossing species is 2.3 times as large as that of selfing species (0.63 and 0.28, respectively; $t_{13} = 4.47$, $P < 0.001$). This result is consistent with the idea that cross-fertilization is predominant in species that maintain considerable genetic load at mutation–selection balance and self-fertilization is predominant

in species that have a relatively small load of lethal and semilethal mutations.

As stated in our introduction, selfing is theoretically expected to reduce the genetic load (genetic purging, Lande and Schemske 1985). Yet low inbreeding depression in selfing species, as observed in our dataset or in Husband and Schemske (1996), do not unambiguously support the genetic purging hypothesis. This is because the observed associations between selfing and inbreeding depression could arise without purging, only because selfing (respectively outcrossing) evolved in lineages that happened to have low (respectively high) inbreeding depression. Several approaches have been taken to document the purging process itself, indirectly by comparing populations with different recent inbreeding histories or directly, by artificially imposing selfing in predominantly outcrossing organisms with initially high inbreeding depression. These approaches have yielded mixed results. Indeed, artificial purging has often succeeded in both plants and animals (Barrett and Charlesworth 1991; Dudash et al. 1997; Lacy and Ballou 1998; Willis 1999; Roff 2002) although indirect approaches usually provide unclear patterns (Byers and Waller 1999). It is also clear from the same studies, both theoretically and empirically, that not all mutations are equally accessible to purging: overdominant polymorphisms and deleterious mutations of weak effects affecting late-expressed traits are not purged as easily as are deleterious mutations with large effects on early-expressed traits. The fact that predominantly selfing plants appear to have reduced genetic loads only for the latter (Husband and Schemske 1996) suggests that historical purging has had some role. Our analysis on animals was restricted to early survival, and thus maximized the opportunity to observe effects of purging; yet when enough data on other fitness components become available, it will be interesting to test whether the same difference between early and late-acting mutations is observed as in plants.

THE WAITING TIME AS A PREDICTOR OF THE MATING SYSTEM IN HERMAPHRODITIC ANIMALS

Preferentially outcrossing species should exhibit a positive waiting time (Tsitroni et al. 2003a). The waiting time is expected to modulate the expected reaction norm of the selfing rate across a range of population densities. As shown in Figure 6, the waiting behavior restricts the occurrence of selfing (and of mixed-mating) to a narrow range of relatively low densities. In agreement with this, in our dataset, outcrossing species exhibit positive and significant waiting times, whereas self-fertilizing species reproduce as soon as individuals are sexually mature (independent of mate availability). Some selfing species even show negative waiting times, probably reflecting a negative effect of pairing, even if restricted to short periods of time. The waiting time is an accurate predictor of the selfing rate and inbreeding depression, as it is strongly correlated with both parameters (Fig. 4B and C).

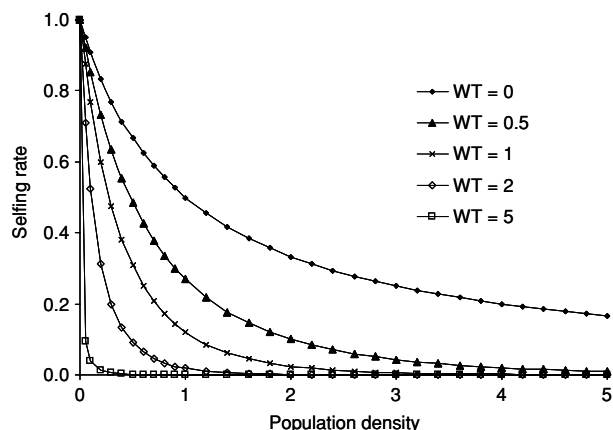


Figure 6. The expected reaction norm of the selfing rate to population density as a function of the waiting time. The selfing rate is computed under the simplest possible model, that is, assuming constant fecundity, mortality and population density, as well as demographic equilibrium, using equations simplified from Tsitroni et al. (2003a). The time scale is chosen so that the mortality rate is 1 (and hence the average life span is 1, and waiting times are expressed in units of the average life span). Density is represented as the rate of mate encounter per unit time (relative to mortality).

Previous intraspecific analyses in the outcrosser *P. acuta* have shown that the age at first reproduction in the absence of mates (T_S) and the survival of the self-fertilized offspring (W_S) are the main drivers of WT and inbreeding depression, respectively (Tsitroni et al. 2003b; Escobar et al. 2007; Escobar et al. 2009). Here, we found the same pattern at the interspecific level. WT is positively correlated with T_S but not with T_O , and AID is negatively correlated with W_S but not with W_O . This suggests that the correlation between WT and AID mainly relies on a correlation between T_S and W_S , and is not affected by the uncertainty in the outcrossed status of offspring of paired individuals.

The overall correlation between WT and AID in our data is largely influenced by the presence of highly selfing species with null WT . Interestingly, however, the correlation persists when we restrict the dataset to preferential outcrossers (especially if WT are expressed in relative units, more comparable across species). This suggests that the WT not only predicts the preferential mating system (nonsignificant WT for selfers, positive WT for outcrossers) but also coevolves quantitatively with inbreeding depression, that is, outcrossing species with high inbreeding depression avoid selfing more strongly, as predicted by the model of Tsitroni et al. (2003a). Such a correlation has already been observed within one species, *P. acuta*, both within and among populations (Escobar et al. 2009). The model of Tsitroni et al. (2003a) predicts that selection on WT switches from stabilizing to directional (toward “infinite” WT) when inbreeding depression approaches 1. In practice “infinite” WT means that isolated individuals would die without ever starting to reproduce. We do observe such individuals

(their impact is quantified by the variable Δ_{max} and can be visualized in Fig. 3) in many outcrossing species. As expected, they are very frequent (and the resulting Δ_{max} are very high) in species with AID very close to 1 (the three *Helisoma* and *P. armigera*).

Large Δ_{max} and/or very high WT characterize species incapable of efficient self-fertilization (*H. anceps*, *H. duryi*, *H. trivolvis*, *P. armigera*, *P. taeniata* and *T. albolabris*; Fig. 3, Tables 2 and 3). Self-incompatibility has not been documented in snails. If it occurs, it would be worthwhile to determine its physiological and genetic basis and, eventually, compare it with the allorecognition system in other invertebrates (Grosberg 1988; Dishaw and Litman 2009) and the well-known self-incompatibility allele system of flowering plants (De Nettancourt 1997). Similarly, we have no information on the physiological basis of the observed waiting behavior; yet, whatever the proximal mechanisms, our data show that waiting times have been lost or acquired repeatedly in association with evolutionary transitions between selfing and outcrossing. We find highly selfing species with no waiting behavior and no apparent inbreeding depression together with outcrossing species with waiting behavior and high apparent inbreeding depression in several independent clades (Planorbidae, Lymnaeidae, and Physidae).

LIMITATIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

In addition to the various limitations already mentioned, the size of our dataset is clearly limited. Gastropod mollusks, especially Basommatophorans, represent most available data and we lack information on important groups such as annelids, ascidians, cestodes, cnidarians, nematodes and trematodes. Based on our limited non-Basommatophoran dataset, the association between selfing rate, waiting time, and apparent inbreeding depression observed in Basommatophorans could be general. In contrast, the bimodality of the distribution of selfing rates is not confirmed outside Basommatophorans but, as mentioned above, the non-Basommatophoran dataset has to be re-evaluated using more robust methods. Globally, we suggest that future studies concentrate on (1) adding species from understudied clades; (2) estimating inbreeding depression over the largest possible proportion of the life cycle and checking the outcrossed status of offspring using molecular markers; (3) estimating selfing rates using robust methods and sampling individuals as early as possible in the life cycle.

COMMON PATTERNS IN MATING-SYSTEM EVOLUTION BETWEEN HERMAPHRODITIC PLANTS AND ANIMALS

Husband and Schemske (1996) demonstrated a negative correlation between inbreeding depression and selfing rate in vascular plants. This correlation was corroborated here in Basommatophorans and other hermaphroditic animals. Our results thus

add support to the theoretical expectation that species evolve toward one of the two divergent states, low inbreeding depression–high selfing rate or high inbreeding depression–low selfing rate (Lande and Schemske 1985; Charlesworth et al. 1990), by testing it in a clade independent from vascular plants. Directional selection of the selfing rate leading to these two evolutionarily stable states seems to accurately describe the macroevolutionary (i.e., among species) patterns of the mating system. This does not mean that mixed-mating is never an evolutionary stable strategy. Several studies have highlighted specific conditions under which mixed mating can be stable (Holsinger 1991; Johnston 1998; Cheptou and Mathias 2001; Porcher and Lande 2005). Yet, the two extreme evolutionary attractors predicted by the disruptive-selection hypothesis seem to account for a large proportion of the global pattern.

We have shown that environmental conditions (e.g., population density) must also be considered in the evolution of selfing rates. We have highlighted, at a larger taxonomic scale than previous studies (Tsitrone et al. 2003b; Escobar et al. 2009), the importance of the waiting time as a life-history trait that summarizes the reaction norm of selfing rates to population density in animals (Fig. 6). To our knowledge, there are no comparable estimates of trait-dependent reaction norms of selfing rates to environmental or population constraints in plants. The waiting behavior in preferentially outcrossing animals is not exactly equivalent to the delayed selfing strategy observed in some plants for several reasons. First, different flowers in the same plant may not have the same pollination history, and delayed selfing may occur in some flowers and not others, resulting in mixed mating at the plant level. Second, in delayed selfing in plants, self-fertilization, but not all reproduction is postponed. Third, pollination is subject to external constraints that are not adequately represented by the density of conspecifics only (e.g., weather, insect activity). All these differences may result in a higher opportunity for “constrained” mixed-mating in plants than in snails. Mixed-mating is indeed surprisingly rare in our snail dataset, much rarer than in plants (see Goodwillie et al. 2005). The accumulation of information on the response of selfing rates to environmental constraints in plants would allow one to interpret this difference, by quantifying the extent to which mixed-mating in plants results from environmental constraints on preferentially outcrossing plants versus from a strategy of producing mixed seed sets in all conditions.

Another interesting aspect lies at the other end of the outcrossing–selfing continuum. Although environmental constraints may explain a substantial fraction of selfing in species that preferentially outcross, it is difficult to imagine external constraints that would impose some outcrossing in preferentially selfing organisms, especially in animals with copulatory behavior, such as snails. A snail only has to avoid copulation or sperm transfer to keep all its ovules for selfing. Why then do we still

observe a positive (often very small) fraction of cross-fertilization in some highly selfing species? It seems hard to believe that this is fortuitous given that these species still exhibit complicated structures (e.g., penial complex and prostate gland) involved in male copulatory behavior. The copulatory behavior itself is maintained, although copulation can be exceedingly rare (copulations have been occasionally witnessed in *B. pfeifferi*, *B. truncatus* and *B. forskalii*). Some of the species may of course be on their way to evolving a complete loss of these characteristics. For instance, many individuals never develop a phallus in *B. truncatus* and several other species (Doums et al. 1998); and simplification of the prostate gland seems to occur in selfing lineages among Lymnaeidae (Jarne et al. 2010). Yet, it seems rather unlikely that most of them are precisely in a transient state. Some outcrossing might be required to cope with variable environmental conditions (Morran et al. 2009b) or it could be the resultant of specific genetic interactions (Charlesworth et al. 1991). It could also be that this residual outcrossing results from conflicting interests of different individuals. If for any reason some individuals have a limited opportunity to invest in the female function (e.g., males in androecious species), they should cross-fertilize as many eggs as they can from other individuals, and therefore impose some outcrossing to other individuals in the population. The latter may be selected to avoid outcrossing, but if it is costly to resist insemination attempts, the result will be an outcrossing rate exceeding the female optimum. In any case, identifying the evolutionary forces that prevent predominantly selfing species from definitively eliminating all opportunities of cross-fertilization is an important goal for the future.

ACKNOWLEDGMENTS

We thank K. E. Knott, F. Prugnolle, Y.-N. Tian-Bi, F. Viard, and S. Weeks for sharing published and unpublished raw molecular and phenotypic data, and two anonymous reviewers for constructive comments. This study received funds from the CNRS, Programme “Ecosystèmes Tropicaux” and ANR JCJC-002 to PD. JSE was funded by the Programme Alban (grant E04D045840CO) and JRA by the National Evolutionary Synthesis Center (NESCent), NSF #EF-0905606.

LITERATURE CITED

- Armbruster, G. F. J., B. Koller, and B. Baur. 2005. Foot mucus and periostracum fraction as non-destructive source of DNA in the land snail *Arianta arbustorum*, and the development of new microsatellite loci. *Conserv. Genet.* 6:313–316.
- Auld, J. R., and R. A. Relyea. 2008. Are there interactive effects of mate availability and predation risk on life history and defence in a simultaneous hermaphrodite? *J. Evol. Biol.* 21:1371–1378.
- Barrett, S. C. H., and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* 352:522–524.
- Barrett, S. C. H., and L. D. Harder. 1996. Ecology and evolution of plant mating. *Trends Ecol. Evol.* 11:73–79.
- Baur, B., and A. Baur. 2000. Social facilitation affects longevity and lifetime reproductive success in a self-fertilizing land snail. *Oikos* 88:612–620.

- Buth, D. G., and J. J. Suloway. 1983. Biochemical genetics of the snail genus *Physa*: a comparison of populations of two species. *Malacologia* 23:351–359.
- Byers, D. L., and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Evol. Syst.* 30:479–513.
- Cain, G. L. 1956. Studies on cross-fertilization and self-fertilization in *Lymnaea stagnalis appressa* Say. *Biol. Bull.* 111:45–52.
- Charbonnel, N., B. Angers, R. Rasatavonjizay, P. Bremond, C. Debain, and P. Jarne. 2002a. The influence of mating system, demography, parasites and colonization on the population structure of *Biomphalaria pfeifferi* in Madagascar. *Mol. Ecol.* 11:2213–2228.
- Charbonnel, N., B. Angers, R. Rasatavonjizay, P. Bremond, and P. Jarne. 2002b. Evolutionary aspects of the metapopulation dynamics of *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni*. *J. Evol. Biol.* 15:248–261.
- Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genet. Res.* 57:177–194.
- Charlesworth, D., and B. Charlesworth. 1979. The evolutionary genetics of sexual systems in flowering plants. *Proc. R. Soc. Lond. B* 205:513–530.
- . 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Evol. Syst.* 18:237–268.
- Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44:1469–1489.
- Chasnov, J. R. 2010. The evolution from females to hermaphrodites results in a sexual conflict over mating in androdioecious nematode worms and clam shrimp. *J. Evol. Biol.* 23:539–556.
- Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L.) (land snail). *Heredity* 71:456–461.
- Cheptou, P. O., and A. Mathias. 2001. Can varying inbreeding depression select for intermediary selfing rates? *Am. Nat.* 157:361–373.
- Costa, M. J. F. S., C. E. Grault, and U. E. C. Confalonieri. 2004. Comparative study of the fecundity and fertility of *Biomphalaria glabrata* (Say, 1818) and *Biomphalaria straminea* (Dunker, 1848) in a laboratory through self-fertilization and cross-fertilization. *Revista do Instituto de Medicina Tropical de São Paulo* 46:157–163.
- Coutellec-Vreto, M. A., A. Guiller, and J. Daguzan. 1994. Allozyme variation in some populations of the freshwater snails *Lymnaea peregra*, *L. auricularia* and *L. stagnalis* (Gastropoda, Pulmonata). *J. Molluscan Studies* 60:393–403.
- Coutellec, M. A., and L. Lagadic. 2006. Effects of self-fertilization, environmental stress and exposure to xenobiotics on fitness-related traits of the freshwater snail *Lymnaea stagnalis*. *Ecotoxicology* 15:199–213.
- David, P., B. Pujol, F. Viard, V. Castella, and J. Goudet. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Mol. Ecol.* 16:2474–2487.
- De Nettancourt, D. 1997. Incompatibility in angiosperms. *Sexual Plant Reproduction* 10:185–199.
- Dillen, L., K. Jordaens, and T. Backeljau. 2009. Life-history variation and breeding system in the hermaphroditic land snail *Succinea putris* (Gastropoda, Pulmonata, Succineidae). *J. Molluscan Studies* 75:311–313.
- Dillon, R. T., C. E. Earnhardt, and T. P. Smith. 2004. Reproductive isolation between *Physa acuta* and *Physa gyrina* in joint culture. *Am. Malacol. Bull.* 19:63–68.
- Dillon, R. T., T. E. McCullough, and C. E. Earnhardt. 2005. Estimates of natural allosperm storage capacity and self-fertilization rate in the hermaphroditic freshwater pulmonate snail, *Physa acuta*. *Invertebrate Reproduction Develop.* 47:111–115.
- Dishaw, L. J., and G. W. Litman. 2009. Invertebrate allorecognition: the origins of histocompatibility. *Curr. Biol.* 19:R286–R288.
- Dole, J. A. 1990. Role of corolla abscission in delayed self-pollination of *Mimulus guttatus* (Scrophulariaceae). *Am. J. Bot.* 77:1505–1507.
- Dolgin, E. S., B. Charlesworth, S. E. Baird, and A. D. Cutter. 2007. Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution* 61:1339–1352.
- Doums, C., P. Bremond, B. Delay, and P. Jarne. 1996a. The genetical and environmental determination of phally polymorphism in the freshwater snail *Bulinus truncatus*. *Genetics* 142:217–225.
- Doums, C., F. Viard, and P. Jarne. 1998. The evolution of phally polymorphism. *Biol. J. Linn. Soc.* 64:273–296.
- Doums, C., F. Viard, A.-F. Pernot, B. Delay, and P. Jarne. 1996b. Inbreeding depression, neutral polymorphism, and copulatory behavior in freshwater snails: a self-fertilization syndrome. *Evolution* 50:1908–1918.
- Dubois, M.-P., A. Nicot, P. Jarne, and P. David. 2008. Characterization of 15 polymorphic microsatellite markers in the freshwater snail *Aplexa marmorata* (Mollusca, Gastropoda). *Mol. Ecol. Res.* 8:1062–1064.
- Dudash, M. R., D. E. Carr, and C. B. Fenster. 1997. Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. *Evolution* 51:54–65.
- Dupuy, V., A. Nicot, P. Jarne, and P. David. 2009. Development of 10 microsatellite loci in the pulmonate snail *Biomphalaria kuhniiana* (Mollusca, Gastropoda). *Mol. Ecol. Res.* 9:255–257.
- Eppley, S. M., P. J. Taylor, and L. K. Jesson. 2007. Self-fertilization in mosses: a comparison of heterozygote deficiency between species with combined versus separate sexes. *Heredity* 98:38–44.
- Escobar, J. S., G. Epinat, V. Sarda, and P. David. 2007. No correlation between inbreeding depression and delayed selfing in the freshwater snail *Physa acuta*. *Evolution* 61:2655–2670.
- Escobar, J. S., B. Facon, P. Jarne, J. Goudet, and P. David. 2009. Correlated evolution of mating strategy and inbreeding depression within and among populations of the hermaphroditic snail *Physa acuta*. *Evolution* 63:2790–2804.
- Escobar, J. S., A. Nicot, and P. David. 2008. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics* 180:1593–1608.
- Fisher, R. A. 1925. *Statistical methods for research workers*. Oliver and Boyd, Edinburgh.
- Goodwillie, C., S. Kalisz, and C. G. Eckert. 2005. The evolutionary enigma of mixed mating in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* 36:47–79.
- Grosberg, R. K. 1988. The evolution of allorecognition specificity in clonal invertebrates. *Q. Rev. Biol.* 63:377–412.
- Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52:696–704.
- Henry, P. Y., and P. Jarne. 2007. Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. *Invertebrate Biol.* 126:138–153.
- Henry, P. Y., L. Bousset, P. Sourrouille, and P. Jarne. 2005. Partial selfing, ecological disturbance and reproductive assurance in an invasive freshwater snail. *Heredity* 95:428–436.
- Henry, P. Y., R. Pradel, and P. Jarne. 2003. Environment-dependent inbreeding depression in a hermaphroditic freshwater snail. *J. Evol. Biol.* 16:1211–1222.
- Holsinger, K. E. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat.* 138:606–622.
- Holsinger, K. E., M. W. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants—a population genetic model. *Am. Nat.* 124:446–453.

- Husband, B., and D. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70.
- Jarne, P., and J. R. Auld. 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. *Evolution* 60:1816–1824.
- Jarne, P., and D. Charlesworth. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annu. Rev. Ecol. Evol. Syst.* 24:441–466.
- Jarne, P., and P. David. 2008. Quantifying inbreeding in natural populations of hermaphroditic organisms. *Heredity* 100:431–439.
- Jarne, P., and B. Delay. 1990. Populations genetics of *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata) in the lake Geneva. *J. Molluscan Studies* 56:317–321.
- Jarne, P., L. Finot, C. Bellec, and B. Delay. 1992. Aphally versus euphally in self-fertile hermaphrodite snails from the species *Bulinus truncatus* (Pulmonata, Planorbidae). *Am. Nat.* 139:424–432.
- Jarne, P., L. Finot, B. Delay, and L. Thaler. 1991. Self-fertilization versus cross-fertilization in the hermaphroditic freshwater snail *Bulinus globosus*. *Evolution* 45:1136–1146.
- Jarne, P., M. A. Perdieu, A. F. Pernot, and P. David. 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.
- Jarne, P., J. P. Pointier, P. David, and J. M. Koene. 2010. Basommatophoran gastropods. Pp. 173–196 in A. Córdoba-Aguilar and J. L. Leonard, eds. *The evolution of primary sexual characters in animals*. Oxford Univ. Press, New York.
- Jarne, P., and T. Städler. 1995. Population genetic structure and mating system evolution in freshwater snails. *Experientia* 51:482–497.
- Jarne, P., M. Vianey-Liaud, and B. Delay. 1993. Selfing and outcrossing in hermaphroditic freshwater gastropods (Basommatophora): where, when and why. *Biol. J. Linn. Soc.* 49:99–125.
- Johnston, M. O. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* 102/103:267–278.
- Jordaens, K., H. De Wolf, N. Van Houtte, B. Vandecasteele, and T. Backeljau. 2006. Genetic variation in two land snails, *Cepaea nemoralis* and *Succinea putris* (Gastropoda, Pulmonata), from sites differing in heavy metal content. *Genetica* 128:227–239.
- Jordaens, K., L. Dillen, and T. Backeljau. 2007. Effects of mating, breeding system and parasites on reproduction in hermaphrodites: pulmonate gastropods (Mollusca). *Anim. Biol.* 57:137–195.
- Kalisz, S., D. Vogler, B. Fails, M. Finer, E. Shepard, T. Herman, and R. Gonzales. 1999. The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *Am. J. Bot.* 86:1239–1247.
- Klips, R. A., and A. A. Snow. 1997. Delayed autonomous self-pollination in *Hibiscus laevis* (Malvaceae). *Am. J. Bot.* 84:48–53.
- Koene, J. M., K. Montagne-Wajer, D. Roelofs, and A. Ter Maat. 2009. The fate of received sperm in the reproductive tract of a hermaphroditic snail and its implications for fertilisation. *Evol. Ecol.* 23:533–543.
- Koene, J. M., and A. Ter Maat. 2005. Sex role alternation in the simultaneously hermaphroditic pond snail *Lymnaea stagnalis* is determined by the availability of seminal fluid. *Anim. Behav.* 69:845–850.
- Lacy, R. C., and J. D. Ballou. 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution* 52:900–909.
- Lande, R., and D. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetics models. *Evolution* 39:24–40.
- Larambergue, M. d. 1939. Etude de l'autofécondation chez les gastéropodes pulmonés: recherches sur l'aphallie et la fécondation chez *Bulinus (Isidora) contortus* Michaud. *Bulletin Biologique de la France et de la Belgique* 73:19–231.
- Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* 113:67–79.
- Lüscher, A., and M. Milinski. 2003. Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: an experimental test of predictions of mixed-mating and Hermaphrodite's Dilemma theory. *J. Evol. Biol.* 16:1030–1037.
- Maddison, W. P., and D. R. Maddison. 2009. Mesquite: A modular system for evolutionary analysis. Version 2.72. Available at <http://mesquiteproject.org> (accessed December 11, 2009).
- Mavárez, J., M. Amarista, J. P. Pointier, and P. Jarne. 2002a. Fine-scale population structure and dispersal in *Biomphalaria glabrata*, the intermediate snail host of *Schistosoma mansoni*, in Venezuela. *Mol. Ecol.* 11:879–889.
- Mavárez, J., J. P. Pointier, P. David, B. Delay, and P. Jarne. 2002b. Genetic differentiation, dispersal and mating system in the schistosome-transmitting freshwater snail *Biomphalaria glabrata*. *Heredity* 89:258–265.
- McCarthy, T. M., and A. Sih. 2008. Relatedness of mates influences mating behaviour and reproductive success of the hermaphroditic freshwater snail *Physa gyrina*. *Evol. Ecol. Res.* 10:77–94.
- McCracken, G. F., and P. F. Brussard. 1980. Self-fertilization in the white-lipped land snail *Triodopsis albolabris*. *Biol. J. Linn. Soc.* 14:429–434.
- Milinski, M. 2006. Fitness consequences of selfing and outcrossing in the cestode *Schistocephalus solidus*. *Integr. Comp. Biol.* 46:373–380.
- Morran, L. T., B. J. Cappy, J. L. Anderson, and P. C. Phillips. 2009a. Sexual partners for the stressed: facultative outcrossing in the self-fertilizing nematode *Caenorhabditis elegans*. *Evolution* 63:1473–1482.
- Morran, L. T., M. D. Parmenter, and P. C. Phillips. 2009b. Mutation load and rapid adaptation favour outcrossing over self-fertilization. *Nature* 462:350–352.
- Murray, J., and B. Clarke. 1966. The inheritance of polymorphic shell characters in *Partula* (Gastropoda). *Genetics* 54:1261–1277.
- Nicot, A., M.-P. Dubois, C. Debain, P. David, and P. Jarne. 2008. Characterization of 15 microsatellite loci in the pulmonate snail *Pseudosuccinea columella* (Mollusca, Gastropoda). *Mol. Ecol. Resources* 8:1281–1284.
- Njiokou, F., C. Bellec, E. K. N'Goran, G. Y. Yapi, B. Delay, and P. Jarne. 1992. Comparative fitness and reproductive isolation between 2 *Bulinus globosus* (Gastropoda, Planorbidae) populations. *J. Molluscan Studies* 58:367–376.
- Njiokou, F., B. Delay, C. Bellec, E. K. N'Goran, G. Y. Yapi, and P. Jarne. 1994. Population genetic structure of the schistosome-vector snail *Bulinus globosus*: examining the role of genetic drift, migration and human activities. *Heredity* 72:488–497.
- Njiokou, F., J. B. Mouafo, T. Félicité, T. Njine, A. S. Ekobo, and P. Jarne. 2000. The influence of self-fertilization and pairing on life-history traits in the freshwater snail *Bulinus forskalii* (Gastropoda, Planorbidae). *Acta Tropica* 76:159–167.
- Pannell, J. R. 2008. Consequences of inbreeding depression due to sex-linked loci for the maintenance of males and outcrossing in branchiopod crustaceans. *Genet. Res.* 90:73–84.
- Paraense, W. L., and L. R. Corrêa. 1988. Self-fertilization in the freshwater snails *Helisoma duryi* and *Helisoma trivolvis*. *Memorias do Instituto Oswaldo Cruz* 83:405–409.
- Porcher, E., and R. Lande. 2005. Loss of gametophytic self-incompatibility with evolution of inbreeding depression. *Evolution* 59:46–60.
- Prugnolle, F., A. Théron, J. P. Pointier, R. Jabbour-Zahab, P. Jarne, P. Durand, and T. De Meeûs. 2005. Dispersal in a parasitic worm and its two hosts: consequence for local adaptation. *Evolution* 59:296–303.
- Puurttinen, M., K. E. Knott, S. Suonpää, K. Nissinen, and V. Kaitala. 2007. Predominance of outcrossing despite low apparent fitness costs of self-fertilization. *J. Evol. Biol.* 20:901–912.

- Richardson, T. E., and A. G. Stephenson. 1989. Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. *Am. J. Bot.* 76:532–538.
- Roff, D. A. 2002. Inbreeding depression: tests of the overdominance and partial dominance hypotheses. *Evolution* 56:768–775.
- Ronfort, J., and D. Couvet. 1995. A stochastic model of selection on selfing rates in structured populations. *Genet. Res.* 65:209–222.
- Schärer, L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution* 63:1377–1405.
- Schemske, D., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52.
- Schjørring, S. 2004. Delayed selfing in relation to the availability of a mating partner in the cestode *Schistocephalus solidus*. *Evolution* 58:2591–2596.
- Schjørring, S., and I. Jäger. 2007. Incestuous mate preference by a simultaneous hermaphrodite with strong inbreeding depression. *Evolution* 61:423–430.
- Städler, T., M. Loew, and B. Streit. 1993. Genetic-evidence for low outcrossing rates in polyploid fresh-water snails (*Ancylus fluviatilis*). *Proc. R. Soc. Lond. B* 251:207–213.
- Tian-Bi, Y. N., E. K. N'Goran, S. P. N'Guetta, B. Matthys, A. Sangare, and P. Jarne. 2008. Prior selfing and the selfing syndrome in animals: an experimental approach in the freshwater snail *Biomphalaria pfeifferi*. *Genet. Res.* 90:61–72.
- Tsitrone, A., A. Duperron, and P. David. 2003a. Delayed selfing as an optimal mating strategy in preferentially outcrossing species: theoretical analysis of the optimal age at first reproduction in relation to mate availability. *Am. Nat.* 162:318–331.
- Tsitrone, A., P. Jarne, and P. David. 2003b. Delayed selfing and resource reallocations in relation to mate availability in the freshwater snail *Physa acuta*. *Am. Nat.* 162:474–488.
- Tuan, R., and L. C. G. Simões. 1998. Effect of self-fertilization on *Biomphalaria tenagophila* (Orbigny, 1835) (Pulmonata: Planorbidae). *Genet. Mol. Biol.* 21. Available at http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47571998000400012&lng=en&nrm=iso (accessed February 6, 2007).
- Uyenoyama, M., K. E. Holsinger, and D. Waller. 1993. Ecological and genetic factors directing the evolution of self-fertilization. *Oxford Surv. Evol. Biol.* 9:327–381.
- Van Duivenboden, Y. A. 1983. Transfer of semen accelerates the onset of egg-laying in female copulants of the hermaphrodite freshwater snail, *Lymnaea stagnalis*. *Int. J. Invert. Reprod.* 6:249–257.
- Verhoeven, K. J. F., K. L. Simonsen, and L. M. McIntyre. 2005. Implementing false discovery rate control: increasing your power. *Oikos* 108:643–647.
- Vianey-Liaud, M., H. Nassi, F. Lancastre, and J. Dupouy. 1989. Duration of pairing and use of allosperm in *Biomphalaria glabrata* (Gastropoda, Planorbidae). *Memorias do Instituto Oswaldo Cruz* 84:41–45.
- Viard, F., C. Doums, and P. Jarne. 1997. Selfing, sexual polymorphism and microsatellites in the hermaphroditic freshwater snail *Bulinus truncatus*. *Proc. R. Soc. Lond. B* 264:39–44.
- Wang, L., H. Zhang, L. Song, and X. Guo. 2007. Loss of allele diversity in introduced populations of the hermaphroditic bay scallop *Argopecten irradians*. *Aquaculture* 271:252–259.
- Weeks, S. C., B. R. Crosser, R. Bennett, M. Gray, and N. Zucker. 2000. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: estimates of inbreeding depression in two populations. *Evolution* 54:878–887.
- Weeks, S. C., V. Marcus, and B. R. Crosser. 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. *Evolution* 53:472–483.
- Weeks, S. C., and N. Zucker. 1999. Rates of inbreeding in the androdioecious clam shrimp *Eulimnadia texana*. *Can. J. Zool.* 77:1402–1408.
- Wethington, A. R., and R. T. J. Dillon. 1991. Sperm storage and evidence for multiple insemination in a natural population of the freshwater snail *Physa*. *Am. Malacol. Bull.* 9:99–102.
- . 1997. Selfing, outcrossing and mixed mating in the freshwater snail *Physa heterostropha*: lifetime fitness and inbreeding depression. *Invertebrate Biol.* 116:192–199.
- Willis, J. H. 1999. Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. *Genetics* 153:1885–1898.
- Wirth, T., A. Baur, and B. Baur. 1997. Mating system and genetic variability in the simultaneously hermaphroditic terrestrial gastropod *Balea perversa* on the Baltic island of Öland, Sweden. *Hereditas* 126:199–209.
- Wolfram, S. 1996. *The mathematica book*. Cambridge Univ. Press, Cambridge, UK.
- Zhang, G., H. Zheng, X. Liu, and X. Guo. 2007. Divergent selection for growth within one self-fertilized line of bay scallop *Argopecten irradians* Lamarck (1819). *Aquaculture* 272:S320.

Associate Editor: J. Pannell

Supporting Information

The following supporting information is available for this article:

Figure S1. Phylogeny inferred with the mitochondrial *cytochrome oxidase I* gene for most Basommatophoran species studied here (model GTR + G + I; gamma shape parameter = 0.532; proportion of invariant sites = 0.400).

Figure S2. Distribution of the waiting time and apparent inbreeding depression among Basommatophorans and non-Basommatophorans.

Table S1. Values of the selfing rate (S) (\pm SD across populations) obtained with the multilocus maximum-likelihood method and F_{is} when both are available.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.