

ANIMALS MIX IT UP TOO: THE DISTRIBUTION OF SELF-FERTILIZATION AMONG HERMAPHRODITIC ANIMALS

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Abstract.—Excluding insects, hermaphroditism occurs in about one-third of animal species, providing numerous opportunities for the evolution of selfing. Here we provide an overview of reproductive traits in hermaphroditic animal species, review the distribution of selfing rates in animals, and test for ecological correlates of selfing. Our dataset (1342 selfing-rate estimates for 142 species) is 97% based on estimates derived from the analysis of population structure (F_{IS} -estimates) using genetic markers. The distribution of selfing is slightly U-shaped and differs significantly from the more strongly U-shaped plant distribution with 47% of animal t -estimates being intermediate (falling between 0.2 and 0.8) compared to 42% for plants. The influence of several factors on the distribution of selfing rates was explored (e.g., number of populations studied per species, habitat, coloniality, sessility, or fertilization type), none of which significantly affect the distribution. Our results suggest that genetic forces might contribute to the evolution of self-fertilization to the same extent in animals and plants, although the high proportion of intermediate outcrossing suggests a significant role of ecological factors (e.g., reproductive assurance) in animals. However, we caution that the distribution of selfing rates in animals is affected by various factors that might bias F_{IS} -estimates, including phylogenetic underrepresentation of highly selfing and outcrossing species, various genotyping errors (e.g., null alleles) and inbreeding depression. This highlights the necessity of obtaining better estimates of selfing for hermaphroditic animals, such as genotyping progeny arrays, as in plants.

Key words.—Animal, hermaphrodite, mating system, population genetic structure, progeny-arrays, self-fertilization.

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A hermaphrodite can be defined as an individual able to produce male and female gametes in its lifetime. This is a surprisingly common situation in multicellular organisms, especially among plants and animals (see Darwin 1876; Bell 1982; Charnov 1982), and the evolution of hermaphroditism to/from separate sexes remains an important evolutionary question (e.g., Ghiselin 1969; Charnov 1982; Ashman 2002). When the production of male and female gametes overlaps in time, at least partly, hermaphrodites are called simultaneous. Simultaneous hermaphroditism opens the way to self-fertilization (henceforth, selfing), in which fertilization involves the male and female gametes from a single individual. (Note that genetic selfing may occur when mating involves male and female individuals from a single clone in asexual species, e.g., in aphids. This situation will not be considered here). Understanding the evolution of the selfing rate is a central question in evolutionary biology (Darwin 1876), which has received widespread attention (see review in Jarne and Charlesworth 1993; Uyenoyama et al. 1993; Barrett 2002; Goodwillie et al. 2005).

Our current understanding of selfing evolution relies on both genetic and ecological forces. Genetic models, initiated by Fisher (1941), assume interplay between the automatic advantage of selfing and inbreeding depression (Lande and Schemske 1985). They generally predict evolution toward pure selfing or pure outcrossing, as a result of disruptive selection disfavoring intermediate selfing rates (i.e., mixed mating). In this way, genetic models of mating system evolution explore the way that the benefits of selfing (i.e., automatic genetic transmission advantage) and costs of selfing (i.e., inbreeding depression) interact to influence the evolu-

tion of the selfing rate. In ecological models, on the other hand, forces external to the genetic costs and benefits of selfing (e.g., pollinator failure, male gamete discounting) may lead to intermediate selfing rates (Jarne and Charlesworth 1993; Uyenoyama et al. 1993; Goodwillie et al. 2005). This is, in fact, true of all models in which the advantage of selfing decreases as the selfing rate increases (Maynard Smith 1978). However ecological models do not account, in general, for the evolution of inbreeding depression with the selfing rate. The prediction of disruptive selection, derived from genetic models, has been tested using empirical distributions of selfing rate estimates among plant species (Schemske and Lande 1985; Vogler and Kalisz 2001). The plant dataset has recently been updated to include 345 species (Goodwillie et al. 2005). The distribution based on this dataset is U-shaped with more species displaying high outcrossing rates than high selfing rates (see fig. 1 in Goodwillie et al. 2005). These two groups indeed represent 60% of the species studied, and their evolution might be described well by genetic models. One explanation for the other 40% is that these species all are on their way toward one end of the equilibrium or the other. It might be more parsimonious to call for an ecological explanation. Indeed, biotically pollinated species comprise 85% of these intermediate selfers, (Goodwillie et al. 2005). Understanding the forces that generate these distributions is therefore of great interest for our understanding of the evolution of selfing rates.

Less is known about selfing in metazoans (i.e., the monophyletic group including all animals; Lecointre and Le Guyader 2001; Eernisse and Peterson 2004). Whether selfing occurs in some animal groups remains unclear, and this topic

has not been reviewed thoroughly. The limited information available from Jarne and Charlesworth (1993) and Jarne (1995) and reviews of a few animal groups (e.g., Heller [1993] and Jarne et al. [1993] for molluscs; Carlon [1999] for corals) suggests that selfing occurs in a few animal groups, the most prominent of which are the cnidarians, trematodes, molluscs, and ascidians. There is evidence that selfing occurs in natural populations, from highly selfing to highly outcrossing species, but a distribution of selfing rates has not been derived. Therefore, we endeavor to review the evolution of hermaphroditism and selfing among animals.

Our objectives are to review the distribution of hermaphroditism, describe how/where self-fertilization occurs among animal phyla, and statistically explore the distribution of selfing rates in animals. We compile genetic studies that estimate the inbreeding coefficient (F_{IS}) from analysis of population structure with the few studies that derive estimates of selfing from progeny-arrays (as described by Ritland 2002). It could be predicted, based on genetic models (e.g., Lande and Schemske 1985) that the distribution of t -estimates will be bimodal, with most species exhibiting high selfing rates ($t < 0.2$) or high outcrossing rates ($t > 0.8$). It was therefore compared to a uniform distribution, as well as to the plant distribution presented in Goodwillie et al. (2005). We also evaluate the influence of various factors that might affect ecological aspects of selfing (e.g., habitat type and fertilization mode). Potential sources of bias in the empirical animal distribution are also discussed.

MATERIALS AND METHODS

The distribution of hermaphroditism among animals described in Jarne and Charlesworth (1993) was updated for the present work. The main sources we used are the series edited by Giese and Pearse (1974, 1975a,b, 1977, 1979), and Adiyodi and Adiyodi (1983a,b, 1989, 1990, 1993, 1994), as well as Bell (1982), Nielsen (1995) and Lecointre and Le Guyader (2001). The primary literature was also used when detailed information is available. Because the animal phylogeny is not fully resolved (see Cracraft and Donoghue 2004), we used the molecular phylogeny presented in Lecointre and Le Guyader (2001), which contains 34 phyla (Table 1) and a conservative estimate of more than 1.2 million species. The numbers of species given in Table 1 should be taken as our currently available best estimate, but not definitive. The occurrence of hermaphroditism was determined for each phylum. In phyla harboring at least one hermaphroditic species, we retrieved information on traits related to reproduction, including the occurrence of asexual reproduction, gonad structure (existence, male and female gametes produced in separated gonads or in an ovotestis), fertilization mode (external or internal), and occurrence of self-fertilization. It should be noted that extensive information on self-fertilization is available only for a few phyla (e.g., molluscs, trematodes, and cnidarians). For the rest of animal phyla, this information is scant; even the reproductive cycle is not fully known in some phyla.

Information on selfing rates per species was derived from extensive literature searches using a bibliographic database (ISI Web of Knowledge) over the period 1975–2005. Key-

TABLE 1. Animal phyla (names after Nielsen 1995) ordered as in Lecointre and Le Guyader (2001). N is the estimated number of species (after Lecointre and Le Guyader 2001; $N_{total} = 1,211,577$). Phyla exhibiting no hermaphroditic species are in bold. Note that phyla 1–4 form the paraphyletic Porifera group, 5–33 are the eumetazoans, 8–33 are the bilaterians, 8–29 are the protostomians (lophotrochozoans, chaetognaths and cuticulates), 8–19 are the lophotrochozoans, and 21–29 are the cuticulates. The cephalochordata and myxinoides (in between 33 and 34) are not represented. Echiurans, pogonophorans and vestimentifera (all gonochoric) are included in the annelids. Gnathostomulids were also included in the annelids, but this is hotly discussed.

Number	Phyla	N
1	Placozoa	1
2	Demosponges	8000
3	Hexactinellid sponges	1000
4	Calcareous sponges	1000
5	Cnidaria	9000
6	Ctenophora	100
7	Myxozoa	1200
8	Rotifera	1800
9	Acanthocephala	1150
10	Cycliophora	1
11	Entoprocta	150
12	Platyhelminthes	13,780
13	Nemertini	900
14	Mollusca	117,495
15	Sipuncula	320
16	Annelida	14,360
17	Ectoprocta (bryozoa)	4500
18	Brochiopoda	335
19	Phoronida	15
20	Chaetognatha	100
21	Gastrotricha	430
22	Onychophora	80
23	Tardigrada	600
24	Arthropoda (euarthropodes)	956,414
25	Nematoda	20,000
26	Nematomorpha	325
27	Kinorhyncha	150
28	Loricifera	9
29	Priapula	16
30	Mesozoa	50
31	Echinodermata	6000
32	Hemichordata	85
33	Urochordata	1300
34	Vertebrata	50,911

words included combinations of phyla names or more vernacular terms such as slug, leech, or worm, and terms related to population genetic structure (e.g., allozyme, F -statistics, heterozygote deficit) and to self-fertilization (e.g., hermaphrodite, selfing, self-incompatibility). More specific searches were also performed within groups, using genus or species names. References tabulated in Jarne (1995) were also used. Studies with one of two types of genetic estimates of the selfing rate were included. First, F_{IS} is related to the selfing rate (S) or the outcrossing rate ($t = 1 - S$) by the classical formula (see e.g., Hartl and Clark 1997):

$$F_{IS} = \frac{S}{2 - S} = \frac{1 - t}{1 + t}. \tag{1}$$

This is at inbreeding equilibrium in an infinite population in which selfing is the sole source of inbreeding. Outcrossing occurs at random, and there is no selection. In practice, forces other than inbreeding may contribute to positive values of

F_{IS} when using genetic markers, including the Wahlund effect, biparental inbreeding, various technical problems (e.g., null alleles), and some forms of selection (e.g., underdominance). Note that in this survey F_{IS} could not be estimated when $H_e = 0$, that is, when no variation occurred within populations. If the authors did not calculate F_{IS} , we calculated the inbreeding coefficient, F_{is} , from the observed heterozygosity (H_o) and gene diversity (H_e) using the equation

$$F_{IS} = 1 - \frac{H_o}{H_e} \quad (2)$$

When more than one estimate of F_{IS} exists for a species, we averaged the F_{IS} -derived estimates of t per population to get species estimates, following the methods used by Goodwillie et al. (2005) for plants. Negative F_{IS} values may occur as the result of biological processes (e.g., disassortative mating), but more probably because of sampling effects when F_{IS} is close or equal to zero. This produces t values higher than 1, and these were included in our survey. The resulting animal database overlapped partially, but not entirely, with that of Jarne (1995), because some species included by Jarne (1995) did not have F_{IS} estimates.

The second method for determining selfing rates was from data derived from progeny arrays (Ritland and Jain 1981; Ritland 2002) using dominant, as well as codominant, markers. This approach provides, in principle, higher-quality data than population analyses and allows estimation of variation in selfing rates among families, biparental inbreeding, and other parameters of the mating system. Unfortunately, analyses based on this approach are rare in animals, and we could include only 35 population-level selfing rate estimates. As above, population estimates were averaged to obtain species estimates. To check whether estimates derived from population genetic structure (F estimates) provided estimates close to those obtained from progeny-arrays (PA estimates), a regression was conducted on the few studies in animals for which the two estimates were available. Because both variables are estimated with error (model II regression), a geometric mean regression was conducted (Sokal and Rohlf 1995, pp. 541–544) using Microsoft (Redmond, WA) Excel. The relationship between the two estimates of the selfing rate is mediated by inbreeding depression (Ritland 1990), and the expectation is that the difference between the PA and the F estimates is much larger at low than at high selfing rates.

Previously, t -estimates in plants have been compared to a uniform distribution to test the predicted bimodal distribution from Lande and Schemske's (1985) model by employing a χ^2 goodness-of-fit test on categories of t -estimates (Schemske and Lande 1985; Barrett and Eckert 1990; Barrett et al. 1996). Vogler and Kalisz (2001) advocated displaying the distribution of t -estimates as a continuous variable, arguing that this cumulative distribution is a more accurate way to portray the distribution of t -estimates. We display the t -estimates in animals in both the categorical and continuous way for comparison with plants (Fig. 1). The distribution of selfing estimates in animals was compared to both a uniform distribution and the plant distribution (separating biotically and abiotically pollinated species) using Kolmogorov-Smirnov tests. Plant data used in our comparison were those of Goodwillie et al. (2005). Data from plant subspecies were averaged

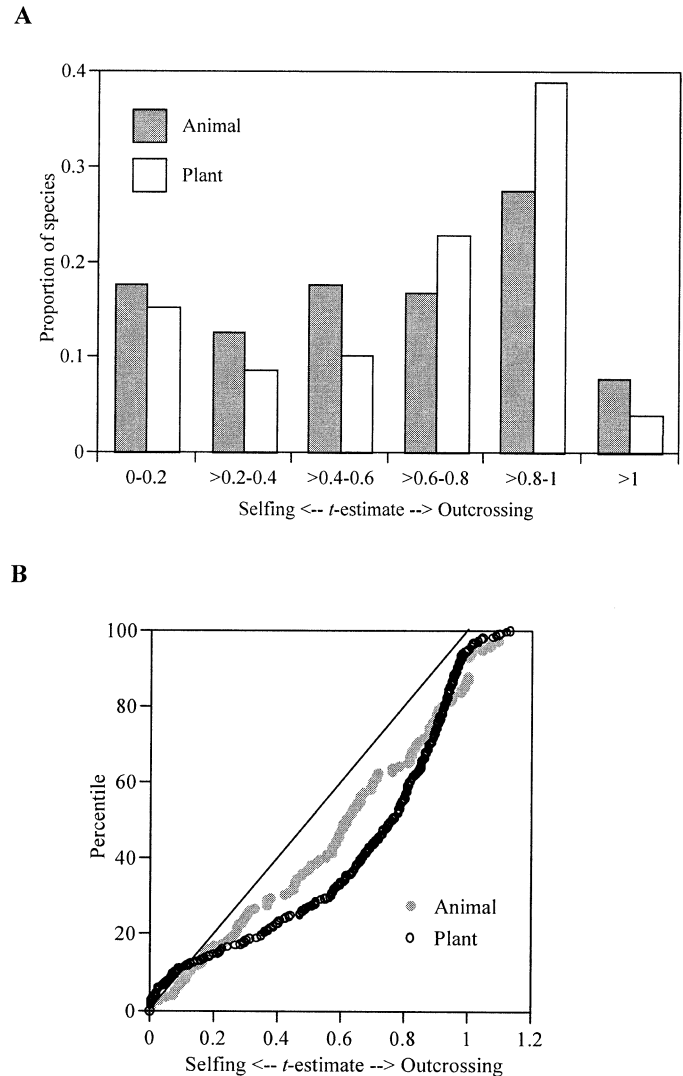


FIG. 1. (A) The distribution of outcrossing rate (t) estimates among hermaphroditic animals ($N = 142$ species) and plants ($N = 342$ species). Plant data are taken from Goodwillie et al. (2005) for comparison. All animal data come from natural populations and were determined using genetic markers (usually allozymes) through progeny arrays or population genetic structure estimates. To compile estimates, multiple samples over time were averaged prior to averaging the population-level estimates. These population-level estimates were then averaged to arrive at species-level estimates of the outcrossing rate ($t = 1 - S$). (B) The cumulative distribution of animal and plant (biotically and abiotically pollinated) t -estimates based on the same datasets. The line is $y = x$ (uniform distribution).

together, and the number of species was 342. Finally, multiple regressions were used to test for the influence of various factors on the selfing rate in hermaphroditic animals. These analyses included three covariables: the number of populations studied per species, the number of individuals per population, and the number of polymorphic loci per population; and four factors: habitat (terrestrial, freshwater, marine, or parasitic species), fertilization type (internal or external), adult sessility (adults sessile or not), and coloniality (species colonial or not). These data were retrieved from the original

TABLE 2. Phyla in which hermaphroditic species were studied and for which we have an estimate of rate of self-fertilization. t is the average outcrossing rate for all species in each phylum (with the standard deviation in parentheses). N is the number of species studied in each phylum.

Phylum	t	N
Annelida	0.882 (0.229)	5
Arthropoda	0.237 (0.096)	2
Cnidaria	0.623 (0.259)	26
Echinodermata	1.000 (0.000)	1
Ectoprocta	0.817 (0.308)	3
Mollusca	0.544 (0.359)	80
Nematoda	0.162 (0.000)	1
Platyhelminthes	0.773 (0.440)	16
Urochordata	0.690 (0.259)	8

articles. Outcrossing rates were arcsine square root-transformed prior to analysis. Multiple regressions were conducted using S-PLUS 6.2 (Insightful 2004). The selfing rate database for animals is available as Appendix 1 (available online only at <http://dx.doi.org/10.1554/06-246.1.s1>).

RESULTS

Hermaphroditism occurs in about 70% of the phyla considered (Table 1), and the 10 phyla in which all species are gonochoric (i.e., dioecious; e.g., Rotifera, Priapulida) account for a limited number of species (about 5000, or ~0.4% of the 1.2 million animal species). However, hermaphroditism is rare to uncommon in many large animal phyla (see Appendix 2 available online only at <http://dx.doi.org/10.1554/06-246.1.s2>), the most prominent of which being Arthropoda. Hermaphroditism is indeed frequent to dominant in 14 phyla, including Porifera, Cnidaria, Platyhelminthes, Mollusca, Urochordata (ascidians), and Annelida. A very rough estimate of the number of hermaphroditic animal species is 65,000, or about 5–6% of animal species (assuming 40% of gastropods are hermaphroditic; see Appendix 2, available online). This fraction increases to about one-third once the arthropods, or even insects, have been removed.

Hermaphroditism does not confer the capacity of self-fertilization. Groups in which selfing might relatively easily evolve are those in which both male and female gametes are produced in a single site/gland (e.g., an ovotestis), as is seen in pulmonate gastropods. However, selfing remains possible even when gametes are produced in separated sites/glands, and when at least male gametes are released into water, a common situation in marine species, such as Porifera, Cnidaria, bivalve molluscs, Bryozoa, and Urochordata (see Appendix 2, available online). The most unlikely situation for the evolution of selfing in hermaphroditic species is probably when self-copulation is required, as in some trematodes or oligochaetes. On the whole, and despite scant information in many taxa, self-fertilization has been reported at least once in all animal phyla displaying some hermaphroditic species (see Appendix 2, available online).

It was possible to estimate the (average) selfing rate in 142 species, or about 0.2% of hermaphroditic species. The average number of populations per species used to estimate species' outcrossing rate was 8.9 (median = 4) and ranged from 1 to 83 (see Appendix 3, available online only at

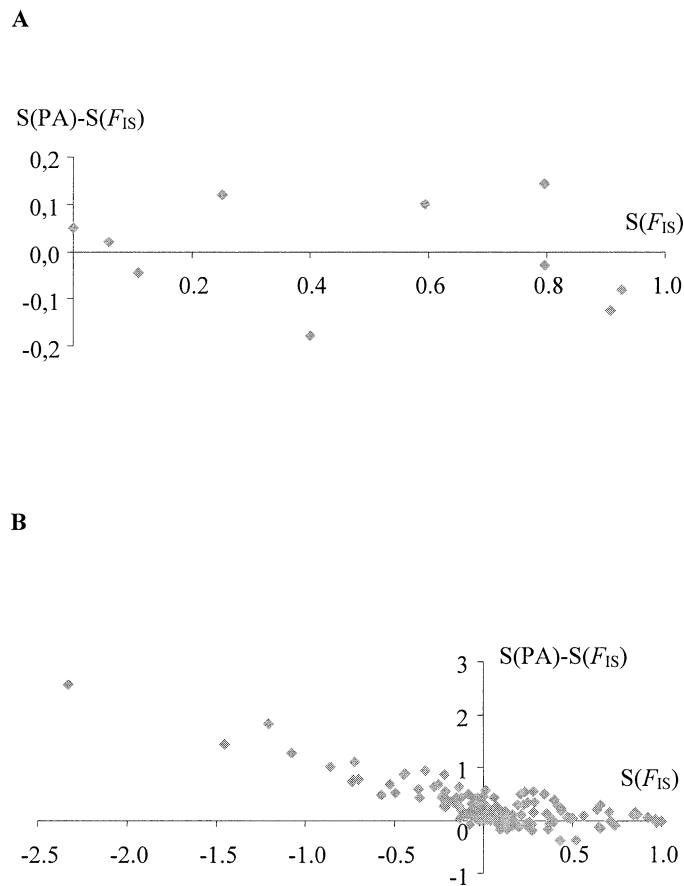


FIG. 2. Difference between the estimate of the selfing rate based on progeny arrays (offspring; S(PA)) and the outcrossing rate based on F_{IS} (estimated in parents; $S(F_{IS})$) as a function of $S(F_{IS})$ in (A) 10 animal species and (B) 149 plant species (database from Goodwillie et al. 2005).

<http://dx.doi.org/10.1554/06-246.1.s3>). Linear modeling of the effect of phylum on the number of populations used to estimate t ($F_{8,129} = 0.956$, $P = 0.474$), the average number of individuals sampled per population ($F_{8,98} = 0.761$, $P = 0.638$), and the average number of polymorphic loci used to estimate population-level t ($F_{6,114} = 0.694$, $P = 0.655$) (and all pairwise interactions; all $P > 0.835$) did not reveal any significant effect. The complete model was not better than a model with no effects ($\chi^2 = 41.5$, $df = 36$, $P = 0.244$). Most data were derived from the study of allozymic variation in natural populations (1106/1141 population estimates; i.e., 97%). It is noticeable, though not surprising, that most estimates were concentrated in a few phyla with large number of species, including Cnidaria (26 species), Mollusca (77), and Platyhelminthes (16) (Table 2; Appendix 3, available online). Estimates of outcrossing rates derived from both population structure and progeny arrays were available in 10 species (all snails; see Appendix 3, available online), and were significantly correlated ($r^2 = 0.913$, $F_{1,8} = 84.2$, $P < 10^{-4}$; Fig. 2A). The estimate of intercept (0.012 ± 0.066) did not differ significantly from zero (t -test, $P = 0.851$). A similar comparison in plants was conducted based on the dataset of Goodwillie et al. (2005). The two estimates were significantly correlated ($r^2 = 0.604$, $F_{1,149} = 226.7$, $P <$

10^{-4}). Geometric mean regression was conducted on the difference between the two estimates as a function of F_{15} -based S (Fig. 2B). A quadratic regression provided a better fit to the data than a linear regression ($r^2 = 0.720$, $F_{1,146} = 191.3$, $P < 10^{-4}$). This quadratic regression curve was a decreasing function of the selfing rate. It cannot be used, though, for predicting values, because we have a model II regression (Sokal and Rohlf 1995, p. 543).

The distribution of animal outcrossing rate is presented in Figure 1, as both histogram and cumulative distribution. The latter significantly differed from a uniform distribution (Kolmogorov-Smirnov test, $Z = 1.740$, $P = 0.005$). It also differs from the whole plant distribution ($Z = 1.709$, $P = 0.006$), and from that of both abiotically pollinated plants ($Z = 2.121$, $P < 0.001$) and biotically pollinated plants ($Z = 1.348$, $P = 0.053$). The whole plant distribution differed from a uniform distribution ($Z = 3.656$, $P < 0.001$), and biotically and abiotically pollinated plant distributions differed as well ($Z = 1.673$, $P = 0.007$), confirming results of Goodwillie et al. (2005) obtained using χ^2 tests.

The distribution of arcsine square root-transformed t -estimates significantly differed from a normal distribution (Shapiro-Wilk test; $W = 0.965$, $P = 0.031$). Exploratory analyses of variance on the four factors and of covariances on the three covariables (see Materials and Methods) did not indicate any significant effects (main effects and pairwise interactions; results not shown). Because the lowest P -value was associated with sessility, the multiple regression included all main effects and pairwise interactions of sessility with the other factors. No significant result was detected, and the overall fit to the data was poor (adjusted $r^2 = -0.001$, $F = 0.989$, $df = 12, 90$, $P = 0.466$). Multiple regressions were also run with phylum as a factor, including (or not) phyla with very small sample size, but no significant effect was detected (results not shown). However, note that a significant difference was observed between molluscs and nonmolluscs ($Z = 1.442$; $P = 0.031$), both distributions differing from a uniform distribution ($Z = 3.074$ and 3.457 for molluscs and nonmolluscs respectively, $P < 0.001$ in both cases). Interestingly, while the distribution is U-shaped in molluscs, the number of species increases with the outcrossing rate in the other group.

DISCUSSION

Hermaphroditism among Animals

Hermaphroditism is extremely common in plants, and only about 6% of species are dioecious (out of 250,000 species; Renner and Ricklefs 1995; Vamosi and Vamosi 2004). Hermaphroditism is thought to be much rarer among animals. Our survey indeed indicates that about 5% of animal species are hermaphroditic. However, this value is much higher (one-third) if the very speciose group of insects is excluded. (Note that insects count for a single [or very rare] event with regard to the evolution of hermaphroditism). Interestingly, the distribution of hermaphroditism across animal phyla (see Appendix 2, available online) requires recurrent evolution between cosexuality and the separate-sex situation at the phylum level. In contrast, hermaphroditism appears to be extremely stable in some groups (e.g., trematoda, pulmonate gastropods), although these groups are extremely old, pre-

sumably originating at or before the Cambrian explosion (see Cracraft and Donoghue 2004). In other groups of similar age, hermaphroditism appears to be more unstable (e.g., Cnidaria, prosobranch gastropods). In the latter group, at least 40 evolutionary shifts to hermaphroditism from the ancestral gonochoric state have occurred (estimated from the data of Heller [1993] and the phylogenies of Tillier et al. 1994; Colgan et al. 2000).

The Distribution of Selfing Rate

Hermaphroditism occurs in a majority of animal phyla, even if sometimes at relatively low frequency. This provides ample opportunity for the evolution of self-fertilization. The distribution of selfing rates among animals is slightly U-shaped—this is also true when considering molluscs only, and indeed differs from a uniform distribution. This is not different from the situation observed in plants (Schemske and Lande 1985; Vogler and Kalisz 2001; Goodwillie et al. 2005) where the distribution is U-shaped (although not symmetric) and differs from a uniform distribution (see above). Even if the animal and plant distributions significantly differ, we note that both have the shape of an asymmetric U, with the high-outcrossing side more frequently represented than the high-selfing side. Taken at face value, this result is not entirely consistent with purely genetic models, which generally predict disruptive selection against mixed mating and, therefore, a completely bimodal distribution (see e.g., Lande and Schemske 1985; Jarne and Charlesworth 1993; Goodwillie et al. 2005). Note that it might technically be possible to adjust a known distribution (e.g., a beta function) to the plant and animal datasets using a maximum likelihood procedure. Notwithstanding the fact that we failed to adjust such a function to the animal data, it is not extremely clear from both genetic and ecological models which function is expected, a concern echoed by Igic and Kohn (2006).

The most widely accepted ecological explanation for the evolution of selfing is the reproductive-assurance hypothesis (Darwin 1876). Under this hypothesis, outcrossing is generally favored, but selfing can evolve if ecological conditions (e.g., mate availability) vary because it is better to self-fertilize than to not reproduce at all (Lloyd 1979, 1992; Holsinger 1996). This hypothesis suggests that selfing rates can vary both within and between populations as a function of mate availability (e.g., population size, sperm limitation) and that mixed mating may be stabilized by variable ecological conditions. Therefore, the observed high frequency of intermediate outcrossing rates in animals is consistent with the reproductive-assurance hypothesis that ecological factors are likely to be involved in mating system evolution. In animals, sperm limitation can occur when individuals do not receive sufficient sperm to fertilize all their eggs as a consequence of low density of individuals. Indeed, fecundity can be limited by sperm availability in marine free-spawners (Levitan and Petersen 1995; Yund 2000). This might result in higher selfing rates, although strategies favoring other aspects of fecundity might evolve as well (e.g., increased life expectancy of male gametes, synchronization of gamete shedding). The occurrence of delayed selfing (Tsitrone et al. 2003a) has been

shown in both freshwater snails (Tsitrone et al. 2003b) and cestodes (Schjørring 2004). This trait shows both variation among individuals and a genetic basis in the freshwater snail *Physa acuta* (Tsitrone et al. 2003b). In sum, these studies and models suggest that mixed mating might well be more than an ephemeral condition before fixation of pure selfing or outcrossing.

To assess the potential role of habitat type and mate availability (fertilization mode, adult sessility, and coloniality) on the evolution of the selfing rate, we evaluated the effects of these factors on the distribution of selfing rates in animals. On the whole, the factors considered in the multiple regression explained a tiny fraction of the variance (none were significant), suggesting that these factors play a very limited role. One possibility is that they represent broad categories (e.g., sessile vs. mobile adults) with a lot of phylogenetic inertia. They might have little relevance for the evolution of selfing because the selfing rate might evolve more rapidly than, for example, adult mobility. In addition, we had no way to quantify population size and its variation in this analysis. If population size varies (as is very likely), it might supersede the effects of habitat type and mate availability. In addition to population size, population structure is likely to be important in the evolution of the selfing rate. Note that increased population subdivision can theoretically stabilize mixed mating by simultaneously decreasing the benefits of selfing (through biparental inbreeding) and the costs of selfing (through the purging of inbreeding depression; Ronfort and Couvet 1995).

Sources of Bias in the Distribution of Selfing Rates

The distribution of selfing rates in hermaphroditic animals reported here might be affected by several sources of bias. The first is a bias in the sampling of phyla (compare Tables 1 and 2; see Appendix 2, available online). Our data represent, to the best of our knowledge, all the currently available information on the selfing rate in hermaphroditic animals. However, the data come from only nine of the approximately 24 phyla (37.5%) exhibiting hermaphroditism. In addition, five of these phyla (Annelida, Arthropoda, Echinodermata, Ectoprocta [Bryozoa], and Nematoda) are represented by less than five species. One phylum (Mollusca) comprises 56% of the dataset. Moreover, the number of species per phylum represented in the database is not correlated with the estimated number of hermaphroditic species per phylum (crudely estimated from Appendix 2, available online; $r = 0.428$, $P = 0.753$). This clear lack of phylogenetic independence in the data can be crudely addressed by determining the average t for the phyla studied (Table 2). The phylum-level mean t -estimates are uniformly distributed (Kolmogorov-Smirnov test, $Z = 0.723$, $P = 0.723$). Note also that, if phylum is included in the multiple regression of outcrossing rate on all factors, the standardized partial regression coefficient is not significant ($P = 0.279$). Clearly, more data, especially from underrepresented phyla (Table 1; Appendices 2 and 3, available online) are required. Goodwillie et al. (2005) made a similar point with regard to the overrepresentation of a few plant families in their dataset, and associated lack of phylogenetic independence. This issue might be less crucial in

animals, because animal phyla are much older than angiosperm families (by presumably up to a factor of five) leaving more time for independent evolution of the selfing rate across species within phyla. However, there is little reason to believe that this bias affects the shape of the distribution of selfing rates.

A second source of bias is underrepresentation of strictly selfing or outcrossing species, a point that likely also affects the plant distribution (Goodwillie et al. 2005; Iqic and Kohn 2006). Self-fertilization is rare, if present, in groups such as the opisthobranch gastropods, polyclad flatworms or some polychaetes (N. Michiels, pers. comm.; see also Appendices 2 and 3, available online), and, therefore, analyses of population genetic structure of selfers are rare. Some hermaphroditic species, such as the earthworm *Lumbricus terrestris* or the terrestrial snail *Cepaea nemoralis*, are likely to be obligate outcrossers despite the capacity for self-fertilization. Highly selfing species are also likely to be underrepresented because they lack appropriate genetic variation for estimating F_{IS} . For example, five species from our initial database did not display any genetic variation, including the snail *Chondrina clienta* (Baur and Klemm 1989) and the fish *Rivulus marmoratus* (Vrijenhoek 1985) suggesting that the number of species in the $t < 0.2$ category is higher than displayed in Figure 1. Moreover, monomorphic populations from 13 selfing species (snails) were not included (Appendix 2, available online), which likely inflated the mean outcrossing rate. A consequence is that the current distribution might appear less U-shaped than it actually is. This, as well as underrepresentation of some phyla, might also explain the peculiar shape of the nonmolluscs' distribution (lack of selfing species).

A third source of bias is that most of the selfing-rate estimates have been derived from F_{IS} -estimates and are therefore affected by a series of technical and/or biological problems. Technically, the methods used for evaluating variation (here, allozymes and microsatellites) often lead to artificially increased F_{IS} -estimates (see Foltz 1986; van Oosterhout et al. 2004; Pompanon et al. 2005). The main source of the problem might be the occurrence of null alleles. Microsatellites also exhibit short-allele dominance and band stuttering. In these three cases, F increases and the bias decreases with increasing selfing rates (van Oosterhout et al. 2006; P. Jarne, unpubl. results). Moreover, null alleles are more likely to be detected at high selfing rates, because of the occurrence of null homozygotes at high frequencies. Estimates of t might also be biased by other sources, including biparental inbreeding, the Wahlund effect, and clonality. Biparental inbreeding leads to overestimating S . Its influence can be approached using the plant dataset from Goodwillie et al. (2005), which contains 154 data points (studies) for which a comparison of t_m and t_s (multi- and single-locus estimates of the selfing rate; see Ritland 2002) can be performed. The mean amount of selfing due to biparental inbreeding ($t_m - t_s$) is 0.034 (SE 0.063), and, interestingly, it is not correlated with t_m ($r = -0.069$, $P = 0.198$), even if biparental inbreeding tends to decrease with inbreeding. Therefore, biparental inbreeding occurs, however S is overestimated by the same amount whatever its value. The Wahlund effect results from sampling individuals from genetically differentiated populations (see e.g., Hartl and Clark 1997), and also generates inflated values

of F_{IS} . However, genetic differentiation among populations of a given species is often a function of geographic distance (isolation by distance), such that populations that are geographically close are genetically very similar. This means that a Wahlund effect is not expected, except when morphologically similar, but genetically divergent entities come into contact, as is often the case in hybrid zones. Clonality, on the other hand, might slightly lower estimates of the inbreeding coefficient (Prugnolle et al. 2005). Indeed, some of the groups considered here show some form of clonal reproduction in their life cycles (Appendixes 2 and 3, available online), but this concerns some Cnidaria and Platyhelminthes species only (see Table 2).

Selfing rates are certainly estimated more accurately using progeny-arrays (Ritland 2002), although this approach might be affected by technical problems as well. This method is also likely to give less biased estimates of selfing rates (at conception), because the time window for the expression of inbreeding depression is much narrower than when using population-structure data. Interestingly, estimates of the selfing rate through both methods are strongly correlated in animals, and the intercept does not differ from zero. This suggests either a limited influence of inbreeding depression, or that the F_{IS} -based estimates are strongly affected by technical problems. The former is unlikely, since ample inbreeding depression has been detected in these species (see e.g., Doums et al. 1996; Jarne et al. 2000). It should also be highlighted that the dataset is limited to 10 species (Fig. 2A). A significant correlation was also found in the much more substantial plant dataset (Fig. 2B), and a quadratic regression provided a rather good fit to data as expected based on the role of inbreeding depression. As mentioned above, this model II regression cannot be used to predict the relationship between the two types of selfing estimates. However, the corresponding model I regression (data not shown) suggests that estimating S from F_{IS} overestimates S by about 20% when $S = 0$, and has no effect when $S > 0.5$. Beyond an incentive for using the progeny-array method as often highlighted (see Ritland 2002), this suggests that the animal distribution is somewhat distorted towards lower selfing rates, especially when S is low. In other words, this distribution might be more U-shaped than reported here. However, the progeny-arrays approach is not always feasible, or is difficult to implement (e.g., in marine or parasitic species). P. David, B. Pujol, F. Viard, V. Castella, and J. Goudet (unpubl. ms.) propose a method for estimating S from multilocus genotypes of a set of individuals—basically the same data used when estimating S from F_{IS} . Although more efficient at large sample sizes, this method is not affected by the technical problems mentioned above, and should be used in future studies.

On the whole, phylogenetic underrepresentation of taxa and technical problems make the distribution of selfing rates displayed here appear less U-shaped than it should, whereas inbreeding depression makes it appear more U-shaped. As pointed out above, biased estimates are more expected in the 0 to 0.5 window of selfing rates (i.e., higher outcrossing rates). Whether these forces equilibrate is far from clear.

Phylogenetic Perspectives

As mentioned above, our analysis is based on a biased sampling of phyla. Moreover, studies within phyla are fo-

cused on a limited number of genera. This certainly calls for enlarging this phylogenetic spectrum, but also points to possible phylogenetic correlations in selfing rates among species within genera (see also Goodwillie et al. 2005; Iqic and Kohn 2006). Such correlations might arise if the evolution of the selfing rate is slow compared to species creation, which seems unlikely. A phylogenetic perspective would also be useful for deepening our understanding of the evolution of selfing rates in animals: an exhaustive phylogeny of a given group (e.g., genus) could be coupled with information on the distribution of selfing among species to test which mating system is ancestral and whether self-fertilization has recurrently evolved. Such an approach has been undertaken in plants (e.g., Kohn et al. [1996], in Pontederiaceae; Bena et al. [1998] in *Medicago*; Armbruster et al. [2002] in Collinsieae). At this point, the conjunction of a good phylogeny and estimates of selfing rates in several species is probably not met, although phylogenies are available in, for example, the snail genus *Biomphalaria* (DeJong et al. 2001). However, we are far from getting an exhaustive picture of the distribution of selfing among *Biomphalaria* species.

Conclusions

Hermaphroditism is widespread among animals, and this has facilitated the evolution of selfing in many animal species. The distribution of selfing rates among animals is similar to that in plants and suggests that similar processes are operating to direct the evolution of selfing. Specifically, both genetic aspects of selfing (transmission advantage and inbreeding depression) and ecological aspects of selfing (reproductive assurance, gamete discounting, and variable mate availability) appear to be important in both animals and plants. However, we clearly need more data before any general conclusions about the evolution of selfing in animals can be reached and the future studies of animals mating systems should employ methods that reduce the bias described above (see Ritland 2002; P. David et al., unpubl. ms).

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