

## Loss of male outcrossing ability in simultaneous hermaphrodites: phylogenetic analyses of pulmonate snails

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(With 1 figure in the text)

Phally, a genital polymorphism found in some species of self-compatible pulmonates (Mollusca: Gastropoda), presents an opportunity to examine factors maintaining outcrossing within an animal species in the presence of recombination. Euphallics, aphyallics, and hemiphallics can self-fertilize but only euphallics develop a functional penis and prostate allowing them to donate sperm. Taxa containing aphyallics and/or hemiphallics are rare within pulmonates (occurring in about 0.3% of species and genera) and are found near the tips of the phylogenetic tree at the species and genus level, suggesting that the loss of male outcrossing ability is unstable and has limited evolutionary potential. Phylogenetic analysis based on parsimony reveals that male outcrossing ability has been lost a minimum of 13 times. We find no unambiguous evidence of reversions from aphyally and/or hemiphally back to pure euphally. In plants, self-fertilization is often associated with habitat and geographic range, and these variables, together with body size, have been hypothesized as factors facilitating the evolution of aphyally. When we control for phylogeny using comparisons of sister taxa, loss of male outcrossing ability is associated with geographic range but not body size or habitat. Furthermore, polyploidy is not associated with the loss of male outcrossing ability, contrary to predictions that low levels of inbreeding depression in polyploids will facilitate the evolution of aphyally and/or hemiphally.

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### Introduction

All other things being equal, alleles increasing the rate of self-fertilization should spread quickly to fixation in organisms which reproduce both by outcrossing and selfing (Nagylaki, 1976; Lloyd, 1979). Consequently, in populations where both modes of reproduction persist, outcrossing must be selectively advantageous (Maynard Smith, 1978). Phally, a genital polymorphism involving the loss of male outcrossing ability, is found in a number of self-compatible hermaphrodite taxa within the Pulmonata (Mollusca: Gastropoda), and presents an opportunity

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to examine factors maintaining outcrossing within an animal species in the presence of recombination. An understanding of the phylogenetic distribution of alternative modes of reproduction and any correlates of such patterns has provided a framework for formulating and testing some of the central hypotheses for the evolution and maintenance of sexual reproduction, parthenogenesis, and mixed mating systems in both plants and animals (e.g. Darwin, 1876; Maynard Smith, 1978; Bell, 1982; Givnish, 1982; Charlesworth, 1985; Harvey & Pagel, 1991). Here we investigate phylogenetic patterns associated with the loss of male outcrossing ability in pulmonate snails.

Some species of self-compatible pulmonates have more than one sexual morph. Euphallic individuals develop fully functional male and female tracts. Hemiphallics develop a reduced penis which is often associated with reduced development of the prostate, vas deferens, and structures associated with formation and storage of calcium darts used in courtship (Watson, 1934; Tompa, 1984). Aphillics do not develop a penis or genital retractor muscle, and distal portions of the male tract (prostate and vas deferens) may be absent or reduced. In both aphallics and hemiphallics, the hermaphrodite gland still produces functional sperm (Geraerts & Joosse, 1984), but there is no anatomical evidence that hemiphallics have a functional penis (Nicklas & Hoffmann, 1981; Tompa, 1984). Consequently, all three morphs are capable of selfing, but aphallics and hemiphallics cannot donate sperm. Regardless of how often aphallics or hemiphallics receive sperm (aphallic *Bulinus truncatus* and *Vertigo* species have been reported to receive sperm [de Larambergue, 1939; Pokryszko, 1990a, b]), selection must favour euphallics where outcrossing is common.

A cost to the growth and maintenance of a full male tract has been detected in the laboratory in *B. truncatus* (Jarne *et al.*, 1992; Schrag & Rollinson, 1994), and presumably exists in other polymorphic species. None the less, in natural populations of species with aphally and/or hemiphally, the proportion of euphallics varies from zero to one (*Acanthinula* [Boycott, 1917a, b]; *Zonitoides* spp. [Watson, 1934]; Vertiginidae [Pokryszko, 1987a]; *Deroceras laeve* [Nicklas & Hoffmann, 1981]; *B. truncatus* [de Larambergue, 1939; Schrag *et al.*, 1994a, b]). The proportion of euphallics can vary seasonally within populations of *Z. nitidus* and *Z. excavatus* (Watson, 1934), *D. laeve* (Nicklas & Hoffmann, 1981) and *B. truncatus* (Schrag *et al.*, 1994b). In some populations of *B. truncatus*, phally is determined by environmental cues during egg and hatchling stages (Schrag & Read, 1992; Schrag *et al.*, 1994b).

Given the cost of growing and maintaining a full male tract, why is euphally maintained within populations of aphallics (and/or hemiphallics) and what factors are associated with the evolution of aphally? A number of competing hypotheses have been developed in the context of within-species investigations. Inbreeding depression may play a role in maintaining outcrossing (e.g. Jain, 1976; Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987; Jarne *et al.*, 1993). However, theoretical and experimental evidence in the case of the allotetraploid, *B. truncatus*, suggests that deleterious effects of inbreeding may not be severe enough to prevent the spread of aphally (Jarne *et al.*, 1992); other reasons why inbreeding depression may be an inadequate explanation of the maintenance of euphallics within *B. truncatus* populations are given elsewhere (Schrag *et al.*, 1994a). Nevertheless, inbreeding depression may be associated with the evolution of phally polymorphisms in other taxa. The magnitude of inbreeding depression depends on natural outcrossing rates, and is likely to be greatest in populations where selfing is rare (Lande & Schemske, 1985). For a given ancestral outcrossing rate, the evolution of polyploidy can decrease inbreeding depression because selfing does not lead to homozygosity as rapidly as in diploids (Lande & Schemske, 1985). In freshwater snail populations, outbreeding is apparently the rule

(Jarne & Delay, 1991). If so, and inbreeding depression is important in preventing the spread of aphally, the loss of male outcrossing ability should be associated with increases in ploidy level.

Probability of mating influences the distribution of selfing and outcrossing in some flowering plants (e.g. Jain, 1976; Clay, 1982; Barrett, Morgan & Husband, 1987; Holtsford & Ellstrand, 1990), and could influence the proportion of euphallics within some pulmonate populations. When the probability of mating (outcrossing) is low, aphally should be favoured because the cost of developing a full male tract is avoided. Under this view, species that often go through population bottlenecks, such as those living in temporary habitats (e.g. ponds which dry up) or those frequently colonizing new areas, are predicted to lose male outcrossing ability. Bottlenecks also purge genetic load, which may decrease inbreeding depression and hence reduce selection against aphally.

Proximate factors such as body size may influence the likelihood of finding a mate. Small size may lead to decreased mobility in snails and, consequently, an association between aphally and small body size has been predicted (Boycott, 1917*a*; Watson, 1934; Riedel, 1953; Pokryszko, 1987*a*). Aphally may also arise in small-bodied taxa because the costs of growing and maintaining a full male tract per unit body mass may be higher due to allometry (Watson, 1934; Riedel, 1953).

Here the phylogenetic distribution of aphally within pulmonate snails is described. We estimate the minimum number of times male outcrossing ability has been lost and investigate whether this is associated with ploidy, habitat, geographic range, and body size.

### Methods

Pulmonate species were dichotomized into taxa where morphs with reduced or absent male tracts have not been reported (here termed 'euphallic taxa') and those where morphs incapable of sperm donation (aphallics or hemiphallics) have been reported (here termed 'polymorphic taxa'). In all cases, conventional syngamy occurs: there is no evidence of parthenogenetic reproduction and aphallics and hemiphallics have a fully functional hermaphrodite gland producing sperm. So far as we are aware, there are no reports of species where euphallics are entirely absent. We do not distinguish between different possible polymorphisms (euphally and aphally; euphally, aphally, and hemiphally; euphally and hemiphally) because morphological evidence suggests that hemiphallics are functionally equivalent to aphallics with respect to male outcrossing ability (Nicklas & Hoffmann, 1981; Tompa, 1984). The terms aphally and hemiphally have been used to describe hermaphrodite snails which lack a penis but are still capable of outcrossing as males. Here we consider only cases of aphally where individuals develop a reproductive anatomy preventing them from sperm donation. For example, some Arionidae lack a penis because other structures are associated with sperm donation, and *Ariolimax* individuals develop a full penis which is bitten off as part of copulation (Tompa, 1984). Similarly, hemiphallic *Heterostoma paupercula* (Helicidae) (Cook & Lace, 1993) apparently have a fully functional penis, but lack the flagellum and epiphallus normally used to make spermatophores in which sperm transfer takes place. As there are other species which outcross without these organs (Cook & Lace, 1993: 381), it is not clear that hemiphallic *H. paupercula* are incapable of sperm donation, so we have taken the conservative view and assumed that they can. In any case, inclusion of this species would not affect our conclusions (it is smaller than related species, has a more restricted geographic range, and is found under volcanic rocks in dry, sparse vegetated areas).

Data on whether taxa are purely euphallic or contain polymorphisms come from reviews by Riedel (1953), Tompa (1984) and Pokryszko (1987*a*) and references therein; full sources are given by Schrag (1993). When information was available for only some species within a given taxon, and each of those species was polymorphic, we assumed that the entire taxon was polymorphic. This will lead to conservative estimates of the number of times polymorphisms have evolved or been lost. Heller (1993) listed genera containing

aphallic species from one of the pulmonate orders we consider (the Stylommatophora), but performed no phylogenetic analysis. Unlike him, we exclude the Arionidae and Thysanophoridae because aphally in these families is not necessarily associated with loss of male outcrossing ability (Riedel, 1953; Tompa, 1984). In addition, we included 2 Stylommatophoran genera where aphally has been reported which Heller (1993) apparently overlooked: *Chondrina* (*C. avenacea* [Riedel, 1953]; *C. clienta* [Pokryszko, 1987a; Baur & Klemm, 1989]) and *Aegopinella* (*A. nitens*; Riedel, 1953).

Phylogeny was inferred from taxonomy since this represents the only phylogenetic hypothesis available for the Gastropoda, given the current state of molluscan systematics (Solem, 1985; Bieler, 1992). Taxonomy follows Wenz (1959–1960) with further resolution taken from Gittenberger (1973) [Chondrinidae], Wiktor (1973) [Limacidae: *Deroceras*], and Brown (1980) [Planorbidae: *Bulinus*]. The tree we derived from this is shown in Fig. 1; the taxonomy is listed in full by Schrag (1993). Estimates of pulmonate species and genera numbers are based on Nicol (1969) and Bouchet & Rocroi (1992).

Ancestral phally states were assigned to the internal nodes of the pulmonate taxonomy using outgroup comparisons and global parsimony methods according to algorithms derived by Maddison, Donoghue & Maddison (1984) and Maddison & Maddison (1992). Polytomies (nodes with more than 2 descendant branches) were treated as areas of ambiguous resolution which could be resolved parsimoniously with respect to phally. If some polytomies actually represent true multiple speciation events, this will lead to conservative estimates of the minimum number of times phally polymorphisms evolved. Furthermore, we assumed that a change from euphally to polymorphism and vice versa was of equal probability. We discuss this assumption further in the **Discussion**.

Lack of independence between species points can bias cross-species tests of adaptive hypotheses (Harvey & Pagel, 1991). Even an approximate knowledge of phylogeny is likely to provide a more appropriate framework for the following analyses than treating species as independent data points, especially when, as below, paired comparisons are used (Felsenstein, 1985; Burt, 1989; Read & Nee, 1995). The method of matched pairwise comparisons (Pagel & Harvey, 1988) controls for similarity due to common ancestry (phylogeny) by analysing associations between traits across independent pairs of sister taxa. Because sister taxa share a common evolutionary history up to the node from which they branch, comparisons of euphallic taxa with their nearest closest relatives with phally polymorphism will reflect evolutionary differences independent of phylogeny. Methods of assuring independence between pairs, in particular with respect to polytomies and cases where data are not available for all pair members, are described by Felsenstein (1988), Burt (1989) and Read (1991).

Sister-taxon comparisons were examined for associations between phally and mean adult body size, habitat, distribution, and ploidy level. Where more than one species was represented in each sister taxon, species values were averaged, following Pagel & Harvey (1988), Burt (1989), Read (1991) and Harvey & Pagel (1991). Two-tailed *P*-values are reported throughout.

## Results

Phally polymorphisms have been reported in 70 of the approximately 47,000 extant species and 16 out of 4075 extant named genera of pulmonates (full listings of polymorphic species are given in Schrag, 1993). Polymorphisms occur near the tips of the phylogeny, at the level of genus or species and once at the level of monogeneric subfamily (Valloniinae) (Fig. 1). In one case, it is formally possible that polymorphisms are maintained at a level between subfamily and family: an equally parsimonious reconstruction of areas of ambiguous resolution in the Acanthinulinae assigns loss of male outcrossing ability as primitive and euphally as derived within that taxon.

Depending on how we resolved regions of ambiguity (equally parsimonious reconstructions) in the Valloniidae and Truncatellininae, the minimum number of times phally polymorphisms evolved from the ancestral state of pure euphally ranged from 13 to 16 (Fig. 1). In all cases, where the most parsimonious reconstruction within a taxon assigns euphally as a derived state, there are

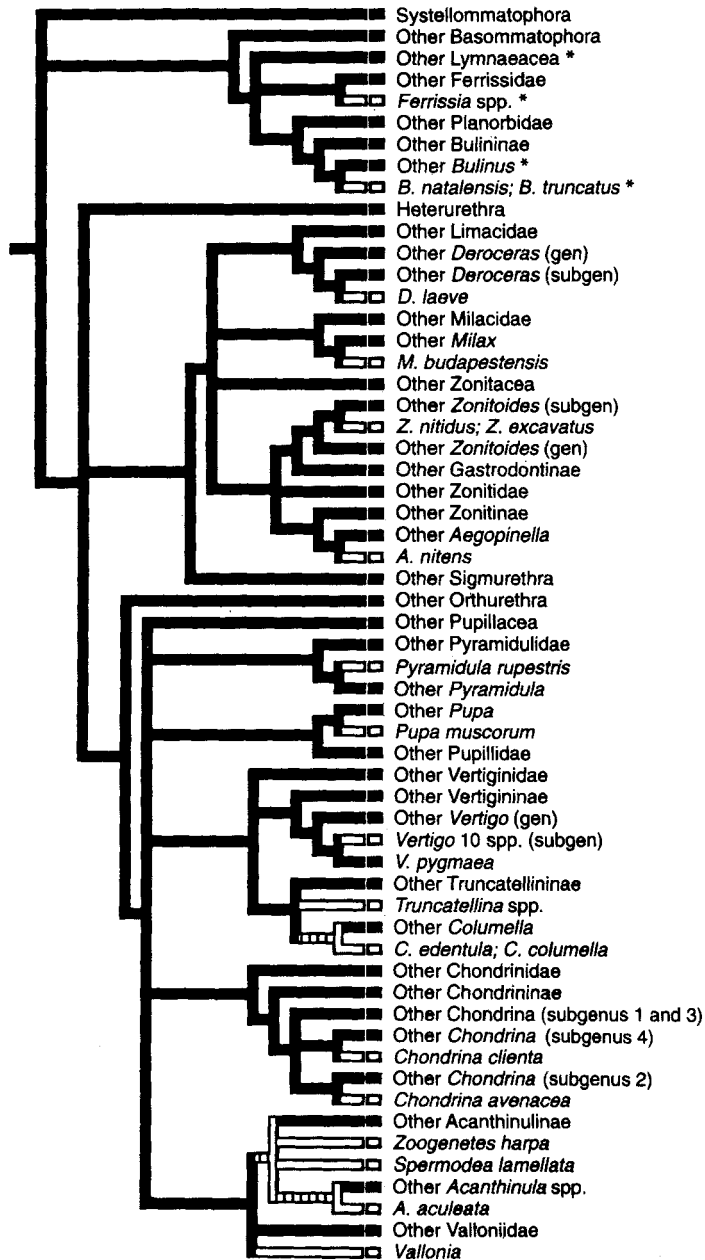


FIG. 1. Evolution of phally polymorphisms in the Pulmonata (Mollusca: Gastropoda). Ancestral phally states were assigned according to parsimony methods described in the text. The term 'other' is used to represent all species in that taxon not shown in lower nodes. Branch length is arbitrary. Unfilled bars and branches = taxa containing aphally and/or hemiphally. Filled bars and branches = purely euphallic taxa. Hatched branches = areas of ambiguous resolution of character state. \* = Taxa containing polyploid species.

TABLE I

Sister-taxon pairs used in matched pairwise comparisons of taxa where loss of male outcrossing ability has been reported (polymorphic taxa) and taxa where it has not (euphallic taxa). Sister taxa for which comparative information was available were chosen as pairs based on the phylogenetic hypothesis in Fig. 1, according to the method described in the text

Pair	Polymorphic taxon	Euphallic taxon
1	<i>Vertigo pusilla</i> <i>V. substriata</i> <i>V. antivertigo</i>	<i>Vertigo pygmaea</i>
2	<i>Columella columella</i> <i>C. edentula</i>	<i>Columella aspera</i>
3	<i>Chondrina avenacea</i>	<i>Chondrina kobelti</i>
4	<i>Chondrina clienta</i>	<i>Chondrina ascendens</i> <i>C. centralis</i>
5	<i>Pupilla muscorum</i>	Other Pupillidae (7 spp.)
6	<i>Aegopinella nitens</i>	<i>Aegopinella nitidula</i> <i>A. minor</i> <i>A. pura</i> <i>A. ressmanni</i>
7	<i>Zonitoides nitidus</i> <i>Z. excavatus</i>	Other Zonitidae (20 spp.)
8	<i>Milax budapestensis</i>	<i>Milax sowerbyi</i> <i>M. gagates</i>
9	<i>Deroceras laeve</i>	<i>Deroceras sturanyi</i>
10	<i>Bulinus truncatus</i> <i>B. natalensis</i>	<i>Bulinus tropicus</i>
11	<i>Ferrissia</i>	<i>Burnupia</i>

equally parsimonious reconstructions with aphyllally as derived. Thus, there is no unambiguous evidence of aphyllally being maintained in a taxon which itself spawns a purely euphallic daughter taxon.

Because phally polymorphisms have arisen a minimum of 13 times in the pulmonates, a maximum of 13 independent sister-taxon comparisons were possible. However, information was available only for the 11 sister-taxon pairs shown in Table I. Phally polymorphisms were not significantly associated with body size, with aphyllally and/or hemiphally taxa smaller bodied than their most closely related purely euphallic taxon in six sister-taxon comparisons, and larger in three (binomial test,  $P > 0.50$ , with one case of no size difference; Table II). Similarly, no association between phally polymorphisms and habitat was apparent (Table III). Within sister-taxon pairs, habitat rarely varied, although across pairs, habitats ranged from tropical freshwater to alpine. Taxa with phally polymorphisms tended to have larger geographic ranges than their purely euphallic sister taxa (Table III). In nine comparisons, it is possible to determine unambiguously which lineage in each sister-taxon comparison has the bigger average range. In eight of these, it was the polymorphic taxon, significantly more than expected by chance alone (binomial  $P = 0.029$ ). In two other cases (Pairs 1 and 7, Table III), sister taxa had very similar ranges.

TABLE II

Mean adult body size of sister-taxa with and without phally polymorphisms. Tabulated data are averages of the sizes of species listed in Table I. E = purely euphallic taxa; P = polymorphic taxa, as described in the text

Pair	Polymorphic taxon	Euphallic taxon	Direction of comparison	Reference
1 ( <i>Vertigo</i> )	1.9 × 1.1 mm	2.0 × 1.1 mm	E > P	Kerney, Cameron & Jungbluth, 1979; Pokryszko 1990a, b
2 ( <i>Columella</i> )	3.3 × 1.5 mm	2.4 × 1.4 mm	P > E	Kerney <i>et al.</i> , 1979; Pokryszko, 1987b
3 ( <i>Chondrina</i> subgenus 2)	7 × 2.4 mm	5.3 × 2.3 mm	P > E	Kerney <i>et al.</i> , 1979; Raven, 1986
4 ( <i>Chondrina</i> subgenus 4)	6 × 2.5 mm	6.8 × 2.7 mm	E > P	Kerney <i>et al.</i> , 1979
5 ( <i>Pupilla</i> )	3.5 × 1.7 mm	3.3 × 1.7 mm	P > E	Kerney & Cameron, 1979
6 ( <i>Aegopinella</i> )	9.5 mm long	7.7 mm long	P > E	Kerney <i>et al.</i> , 1979
7 ( <i>Zonitoides</i> )	6.1 mm long	7.6 mm long	E > P	Kerney & Cameron, 1979
8 ( <i>Milax</i> )	50–100 mm	70–80 mm	E ≈ P	Kerney <i>et al.</i> , 1979; Foltz, Ochman & Selander, 1984
9 ( <i>Deroceras</i> )	20 mm	35 mm	E > P	Kerney & Cameron, 1979
10 ( <i>Bulinus</i> )	12.5 mm × 8.5 mm	13 mm × 10 mm	E > P	Brown, 1980
11 ( <i>Ferrissia</i> )	3.5 mm long	5.6 mm long	E > P	Brown, 1980

Polyploidy has arisen independently at least four times in the Pulmonata, all within the Basommatophora (four genera in three families [Patterson & Burch, 1978]; Fig. 1). Consequently, it is possible to examine whether polyploidy predisposes taxa to the loss of male outcrossing ability using four sister-taxon comparisons. In two of these (*Ferrissia* and *Bulinus*), evolutionary transitions to polyploidy were associated with the loss of male outcrossing ability. In each, the chance that aphally should have arisen in the polyploid rather than the diploid lineage is 0.5 under the null hypothesis of no association. The chance that polyploidy and aphally should be associated in both *Ferrissia* and *Bulinus* is thus  $0.5^2 = 0.25$ , far short of conventional significance (the logic of this sort of test and why it is to be preferred over others is discussed at length by Read & Nee, 1993, 1995). We note also that within *Bulinus*, aphally is associated with tetraploids but not with hexaploids or octoploids (Brown, 1980). In the remaining two sister-taxon comparisons involving polyploids (*Ancylus* and *Gyraulus*), all taxa are euphallic. Polyploidy is clearly not a necessary condition for the loss of male outcrossing ability: aphally has arisen at least 11 times in diploid taxa. Thus there is no evidence of an association between ploidy and phally.

### Discussion

Male outcrossing ability has been lost from an ancestral state of pure euphally at least 13 times. In each case, phally polymorphisms have not been associated with large radiations, but rather appear near the tips of the pulmonate phylogenetic tree. Consequently, the trait is relatively rare, with aphallics and/or hemiphallics recorded in only about 0.15% of named pulmonate species. In addition, there is no unambiguous evidence of transitions back to pure euphally.

TABLE III  
*Habitat and distribution of independent pairs of sister-taxa with and without phally polymorphisms*

Pair	HABITAT		DISTRIBUTION			Reference*
	Polymorphic taxon	Euphallic taxon	Polymorphic taxon	Euphallic taxon	Euphallic taxon	
1 ( <i>Vertigo</i> )	Semi-open grassland, marshes	Open, dry grassland, marshes, calcareous substrata	U.K.; Europe; Scandinavia	U.K.; Europe; Scandinavia	U.K.; Europe; Scandinavia	Pokryszko, 1990a, b
2 ( <i>Columella</i> )	Damp, calcareous substrata, grassland and semi-open habitats	Mountains, marshes, pine forest	N, W Europe; N, C Asia	N, W Europe; N Asia	N, W Europe; N Asia	Pokryszko, 1987b
3 ( <i>Chondrina</i> subgenus 2)	Restricted: open limestone, over 2000 m	Restricted: limestone, mountains	S, C Europe; S Asia; Western Palaearctic	S, C Europe; S Asia; Western Palaearctic	Cantabrian and Basque mountains	Raven, 1986
4 ( <i>Chondrina</i> subgenus 4)	Open, rocky, calcareous habitats, mountains	Limestone, rocky mountains	C, SE Europe; Sweden; N, W Africa	C, SE Europe; Sweden; N, W Africa	Pyrenees	Baur & Klemm, 1989
5 ( <i>Pupilla</i> )	Dry, open, calcareous habitats: stone, sand, grass up to 1500 m	Rocks, woods, damp or dry, 300–2800 m	Holarctic	Holarctic	Alpine; W, C Europe	
6 ( <i>Aegopinella</i> )	Woods, rock, mountains, damp habitats up to 2500 m	Damp woodland, ground litter, mountains	S, C Europe; alpine	S, C Europe; alpine	Europe; U.K.; S. Scandinavia	Mordan, 1978



7 ( <i>Zonitoides</i> )	Damp, marshes, ground litter, non-calcareous substrata	Damp, marshes, rocks, mountains, wide range of habitats within species	U.K.; Europe; N. United States; Costa Rica	Alpine; C, S Europe; 1 sp. holarctic	Shikov, 1984
8 ( <i>Milax</i> )	Subterranean; cultivated areas: fields, gardens	Semi-open woodlands, gardens, fields	E, NW Europe; U.S.; Canada; Hawaii; Australia	W, NE U.S.; U.K.; Ireland; Spain	Foltz <i>et al.</i> , 1984; Runham & Hunter, 1970; Quick, 1960
9 ( <i>Deroceras</i> )	Wild and cultivated areas: leaf litter, stones, logs	Damp, cultivated areas	U.S.; W, C Europe; Scandinavia; New Zealand	C, Eastern Europe	Runham & Hunter, 1970; Chichester & Getz, 1969; Shikov, 1984
10 ( <i>Bulinus</i> )	Small pools, lakes, slow flowing rivers, irrigation systems, altitudes less than 2000 m	Small pools, slow flowing rivers, irrigation systems, altitudes between 710 and 2750 m; not found in large lakes or in warmest climatic regions	Africa; Iran; Israel; Sardinia; Sicily; Iberian peninsula	E, S Africa	Brown, 1980; Brown, Shaw & Rollinson, 1991; Schrag <i>et al.</i> , 1994b
11 ( <i>Ferrissia</i> )	Stagnant seasonal pools, irrigation systems, large lakes	Stones in streams or lakes, smaller variety of habitats than <i>Ferrissia</i>	Worldwide; W. Europe; Africa; S and E. Asia; Australia; Oceania	Africa: Ethiopia, N Angola, Zaire, Zimbabwe, Namibia, S. Africa	Brown, 1980

\* Information on habitat and distribution of the Stylommatophora comes from Kerney & Cameron (1979) and Kerney *et al.* (1979); only additional references are listed

Parthenogenesis in multicellular animals is also a rare trait maintained near the tips of the phylogenetic tree, and this pattern has been used as evidence of a group selection advantage to sex (Maynard Smith, 1978, 1989; Bell, 1982). By analogy, the phylogenetic pattern revealed in Fig. 1 suggests that hemiphallics and aphyllics have limited evolutionary potential, with euphallic taxa having an advantage over polymorphic taxa at a group selection level. Stebbins (1957) has argued that self-fertilization restricts the evolutionary potential of plant taxa. Consistent with this, no entirely aphyllic species have been reported even though, within species, euphallics may be absent from some populations (Brown & Wright, 1972; Schrag *et al.*, 1994a, b).

Our data do not, of course, demonstrate that the evolution of aphyllity and/or hemiphally represents an evolutionary dead end for a taxon. It may be that there are frequent reversions to pure euphally. Although we found no unambiguous evidence of such reversions, aphyllity and/or hemiphally may be lost sufficiently rapidly that reversions are undetectable. However, theoretical and empirical observations in plants suggest that, once species evolve high levels of selfing, they are unlikely to revert to outcrossing (Jain, 1976; Maynard Smith, 1989). In any case, the implication is that aphyllity *per se* has limited evolutionary potential.

Simultaneous hermaphroditism and self-compatibility appear to be necessary but not sufficient conditions for the evolution of aphyllity or hemiphally, at least among organisms with a fully functional hermaphrodite gland. This may explain why aphyllity has not been reported in Opisthobranch or Prosobranch gastropods. Only 3% of Prosobranchs are hermaphrodite and, while the vast majority of Opisthobranchs are functional simultaneous hermaphrodites, self-fertilization has been reported in only three species (Tompa, Verdonk & van den Biggelaar, 1984; Heller, 1993). If selfing is not possible, selection (at least on autosomal genes) would favour euphally, unless the costs of sperm donation are sufficiently high. In contrast to these two groups, the ability to reproduce uniparentally is widespread in the Pulmonata (Tompa, 1984; Njiokou *et al.*, 1993; Heller, 1993).

We found no evidence that aphyllity and/or hemiphally evolved more often in small-bodied taxa, or polyploid taxa, or in those occupying more ephemeral habitats, though these variables are often associated with self-fertilization in plants and have been hypothesized as factors facilitating the evolution of aphyllity. Indeed, geographic range, which was typically larger in taxa with phally polymorphisms, was the only factor which correlated significantly with loss of male outcrossing ability. This supports theoretical predictions that selfing species may be better colonizers and survive in a wider range of environments than outcrossing species because of the benefits of reproductive assurance (Jain, 1976). It is also consistent with empirical observations in some plants that selfing species have less restricted geographic ranges than their outcrossing relatives (Jain, 1976; Price & Jain, 1981). Alternatively, if geographic range correlates with the number of individuals or populations in a species, then the chances of aphyllity arising in that species may be higher. Likewise, if distribution correlates with number of populations sampled, it may just be that the chances of observing aphyllic individuals are higher in species with larger distributions.

The wide range of habitat types across sister-taxon pairs (tropical and temperate; land and freshwater; low altitude and high altitude) suggests that any ecological correlates of phally polymorphisms must be those varying within broad habitat categories (Table III). We note that there are no reported cases of aphyllity or hemiphally in marine gastropods. This may simply reflect the rarity of self-compatible gastropods in marine environments (Tompa *et al.*, 1984; Ghiselin, 1987; Heller, 1993) rather than an ecological feature of marine environments *per se*.

Contrary to predictions that low levels of inbreeding depression may facilitate the loss of male

outcrossing ability in polyploids, we found no evidence of a significant association between polyploidy and the loss of male outcrossing ability. Ploidy levels in pulmonates are relatively poorly studied (e.g. only 24 species out of seven families in the Orthurethra have been examined (Patterson & Burch, 1978)), and it may be that additional data will modify this conclusion. Similarly, we found no support for the idea that the evolution of aphyllity is more likely in small-bodied taxa because they are less able to cover distances necessary to find mates (Boycott, 1917a; Pokryszko, 1987a). Adult body size is, however, probably only loosely associated with probability of finding a mate, and a measure taking into account population density and costs of searching and copulation would provide a better test; unfortunately, we know of no data amenable to comparative tests. Across natural populations of *B. truncatus*, proportion of euphallics was not associated with snail density (Schrag *et al.*, 1994a, b).

In *B. truncatus* there is some evidence that, as suggested by Hamilton, Axelrod & Tanese (1990), frequency-dependent selection imposed by parasites may favour outcrossing and hence select against aphyllity (Schrag *et al.*, 1994a, b). This type of hypothesis can in principle be investigated across taxa (e.g. Burt & Bell, 1987; Read, 1991). Burt & Bell (1987) argued that longer-lived mammal species were more subject to parasitism. To the extent that body size in pulmonates correlates with exposure to parasites, we found no support for the idea.

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