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Stability and genetic basis of variability of phally polymorphism in natural populations of the self-fertile freshwater snail *Bulinus truncatus*

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Summary

We investigated the genetic variability for phally polymorphism within and between natural populations of the hermaphrodite self-fertile freshwater snail *Bulinus truncatus*. Phally polymorphism is characterized by the co-occurrence in natural populations of regular hermaphrodite individuals (euphallic) and individuals deprived of the male copulatory organ (aphallic). The two morphs can both self-fertilize and outcross. However, aphallic individuals cannot outcross as males. We examined the variation of the aphally ratio in 22 natural populations from Niger over two successive years. During the second years, populations were sampled three times at 3 week intervals. The aphally ratio was highly variable among populations at a given sampling data and remained relatively stable over time. For 10 of these populations, one population from Corsica and two from Sardinia, we also estimated the between- and within-population variability, analysing the aphally ratio of 346 families under laboratory conditions. The aphally ratio varied significantly among populations and was highly correlated with the aphally ratio of the natural populations. Some within-population variability, associated with a high value of the broad sense heritability, was observed in four populations out of 13. In these populations, aphallic individuals produced significantly more aphallic offspring than euphallic individuals. Our results indicate a strong genetic basis for aphally, with large genetic differences among populations and some genetic variability for aphally within populations. We discuss the adaptive and stochastic factors that may shape the distribution of the genetic variability for aphally.

1. Introduction

The magnitude of genetic variation underlying selected traits determines the potential for selection to act on these traits. To understand the evolution of selfing in hermaphrodites, one therefore has to consider the genetic variability of traits related to the mating system. Most investigations on this topic have focused on plants for which some floral characteristics such as the degree of protandry can easily be related to the selfing rate (Schoen, 1982; Wyatt, 1984; Fenster & Ritland, 1994). In animals, only a few studies have been performed to examine the evolution of selfing (review in Jarne & Charlesworth, 1993) and none has examined the genetic variability of traits related to selfing.

Phally polymorphism, which occurs in some hermaphrodite snails, provides the opportunity to study the evolution of selfing in relation to sexual polymorphisms in animals. Indeed, species exhibiting phally polymorphism are characterized by the co-occurrence of two sexual morphs in natural populations: regular hermaphrodite individuals (euphallic) and aphallic individuals which are deprived of the male copulatory organ (Larambergue, 1939). The two sexual morphs can both self-fertilize and outcross as females. However, aphallic individuals cannot transmit sperm and therefore cannot outcross as male. A major consequence is that selfing is obligatory in strictly aphallic populations. If euphallic individuals mainly outcross, we also expect a correlation between the selfing rate and the aphally ratio (ratio of aphallic over all individuals) as has been assumed by previous studies (Jarne *et al.* 1992; Schrag *et al.* 1994*a*). However, a recent study showed that in the freshwater snail *Bulinus truncatus* the selfing rate is high whatever the aphally ratio (Viard *et al.* 1996).

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Most studies on aphally have been performed in the hermaphrodite self-fertile snail *B. truncatus*. This species has also been intensely studied for its allozyme and DNA polymorphisms, its mating system and population dynamics (review in Brown, 1994). *B. truncatus* is one of the intermediate African hosts of the human-infecting trematode *Schistosoma*. It is an allotetraploid species with a distribution extending from the south of Africa to the Middle East and the Mediterranean islands (Brown, 1994). Both genetic and environmental factors have been shown to determine aphally and they could be responsible for the variability among natural populations. The determination of aphally seems quite complex, the two sexual morphs being able to produce any frequency of aphallic individuals among their offspring (Larambergue, 1939; Doums *et al.* 1996*a*). An individual, whatever its sexual morph, can be characterized by its family aphally ratio, which corresponds to the frequency of aphallic individuals among its offspring (Larambergue, 1939; Doums *et al.* 1996*a*). The aphally ratio increases with temperature both under laboratory conditions (Schrag & Read, 1992; Doums *et al.* 1996*a*) and in natural populations (Schrag *et al.* 1994*b*). The aphally ratio is highly variable among natural populations (Larambergue, 1939; Schrag *et al.* 1994*b*). However, little is known of its temporal stability, and it remains unclear whether the basis of its variability among populations is primarily genetic or environmental.

B. truncatus colonizes different types of freshwater habitats such as rivers, ponds and reservoirs which are generally characterized by large variation in water availability. Some habitats even dry out for many months during the dry season. This can greatly influence the population dynamics of snails (Brown, 1994). It has been suggested that recurrent population bottlenecks as well as self-fertilization reduce neutral genetic variation maintained within populations and modify its distribution among populations (see Jarne, 1995). These factors may also influence the distribution of genetic variation for selected traits, such as aphally, within and between populations.

The genetic variability for aphally has been investigated within only a few of the populations studied for phally polymorphism (Schrag *et al.* 1992, 1994*b*; Doums *et al.* 1996*a*). Analysing the genetic variability over many populations is required, and was the aim of the present study. We specifically address the four following questions: (i) Is the aphally ratio stable over time in natural populations? (ii) Is the variability in the aphally ratio among populations due to genetic differences? (iii) Is there some genetic variation for aphally within natural populations? (iv) Is there an effect of water availability on either the population aphally ratio or the genetic variability for aphally within populations?

2. Materials and methods

(i) *Field collections*

Snails were collected from 22 sites in Niger (see Fig. 1 and Table 1 for population locations and characteristics) between 22 February and 2 March 1994 and in January and February 1995. In 1995, populations were sampled three times at 3 week intervals (sampling dates are given in Table 1). We were not able to sample all populations at all dates because either ponds had dried out or access to the sites was difficult (presence of guerillas or highly degraded trails). Snails were hand-collected by looking for vegetation (mainly water lily) and woody stems over areas of no more than 500 m^2. Only snails over 2·5 mm were collected and brought back to the laboratory. We also collected snails from two Sardinian populations and one Corsican population in September 1993 (Table 2). Phally was scored by observing narcotized individuals (using pentobarbital sodium) under a binocular microscope. Mortality between collection and scoring was very low (about 1%).

(ii) Laboratory survey

From each of 10 populations from Niger and Corsican and Sardinian populations, an average of 30 snails were collected and isolated in 80 ml plastic boxes (one snail per box). Four Niger populations (Taka, Bala, Niumpalma and Doubalma) were studied for two sampling dates (Table 3). Egg capsules laid within 1 week were collected for each snail separately and put in 80 ml plastic boxes. For individuals of the same population, capsule collection was performed during the same week. For all populations, the week of capsule collection occurred within 6 weeks after collection of the adults from natural populations. As the selfing rate in natural populations of *B. truncatus* is very high (around 0·8–1·0: Doums *et al.* 1996*a*; Viard *et al.* 1996), isolated snails in this study probably mainly self-fertilized regardless of the week of egg collection. The sexual morph of offspring from each individual was scored when the shell size of the offspring was larger than 2·5–3·0 mm. The number of individuals (families) per population which produced offspring and the number of offspring checked for sexual morph per population are indicated in Table 3. A mean number of 27 families per population and 11 offspring per family were studied. Overall, phally status was checked for 9289 individuals from natural populations and 3937 individuals of the first laboratory generation. All individuals were reared at a constant temperature of 25 °C (±1 °C) and under a 12/12 h photoperiod. They were fed with boiled lettuce. Water and food were changed twice a week. The position of the rearing boxes was randomized throughout the experiment.

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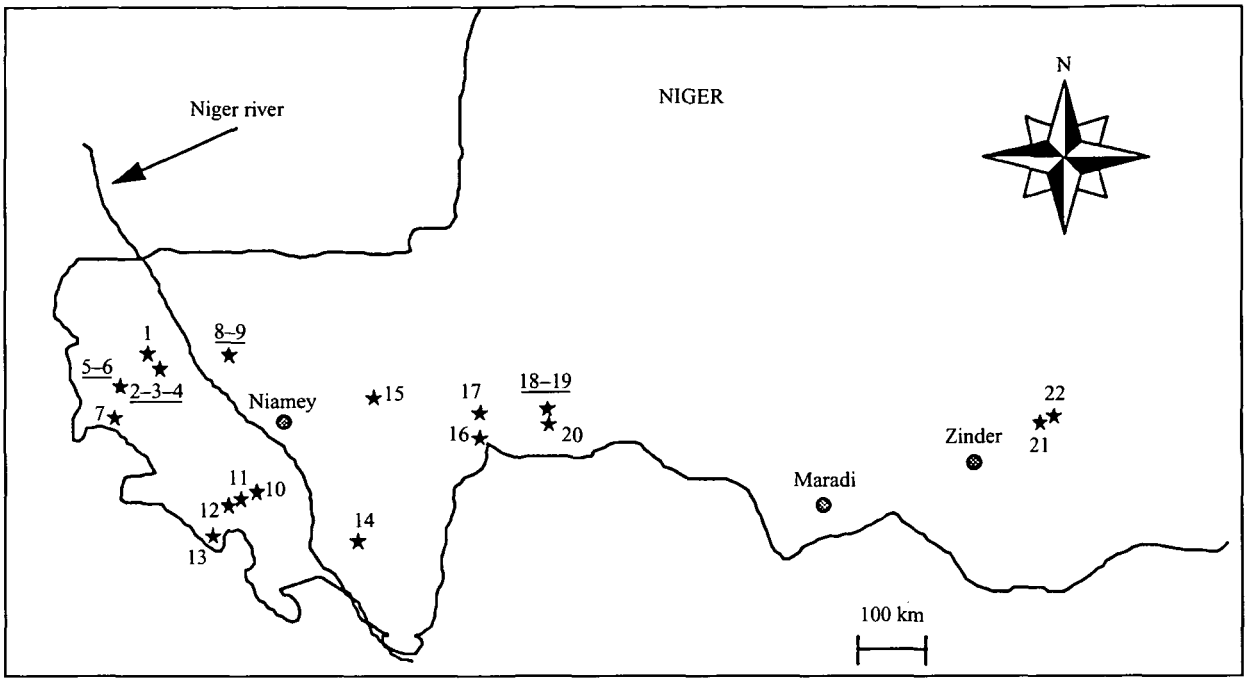

Fig. 1. Location of populations sampled in southwestern Niger. Numbers refer to sites in Table 1. Sampling sites within a pond are underlined (the mean distance between sites within population is about 1·5 km).

Table 1. *Population characteristics and aphally ratio for the four sampling dates in Niger*

			Aphally ratio			
Site	Population	Habitat	1994	1995–1	1995–2	1995–3
1	Kokourou	T	0·67 (116)	—	—	—
2	Namaga PM	S	0·69 (64)	0·77 (44)	0·38 (52)	0·57 (60)
3	**Namaga B**	S	0·53 (192)	0·85 (76)	0·91 (66)	**0·75 (230)**
4	Namaga W	S	0·77 (47)	0·93 (14)	0·66 (65)	0·88 (107)
5	Tera R	P	0·74 (54)	0·86 (100)	0·79 (91)	0·92 (96)
6	Tera D	T	0·54 (13)	0·70 (10)	0·81 (193)	0·72 (213)
7	**Taka**	T	—	**1·00 (136)**	0·94 (345)	**0·98 (378)**
8	**Mari Sud**	S	0·84 (129)	0·75 (224)	0·62 (193)	**0·71 (480)**
9	**Mari Nord**	S	0·76 (37)	**0·84 (237)**	0·78 (201)	0·82 (327)
10	**Kobouri**	P	0·00 (23)	—	—	0·00 (14)
11	**Kotaki**	T	—	**0·69 (228)**	0·48 (830)	0·48 (510)
12	**Bala**	T	0·36 (83)	**0·51 (69)**	0·29 (121)	**0·25 (202)**
13	**Niumpalma**	T	—	0·72 (318)	**0·36 (272)**	**0·19 (188)**
14	Tiamey	P	0·17 (30)	—	—	—
15	Bungario	T	0·72 (25)	—	—	—
16	**Ligido**	T	—	**0·80 (326)**	0·84 (51)	0·77 (52)
17	**Doubalma**	T	0·75 (12)	**0·74 (176)**	0·68 (160)	**0·59 (254)**
18	**Boyze I**	P	0·88 (102)	**0·81 (69)**	0·69 (23)	0·88 (52)
19	Boyze II	P	0·94 (165)	0·98 (59)	0·96 (28)	—
20	Yaya	T	0·80 (10)	0·80 (15)	—	—
21	Mada	T	1·00 (59)	—	—	—
22	Bouktra	T	0·89 (19)	—	—	—

Site numbers correspond to those in Fig. 1. Sites where snails were collected for laboratory experiments (see Section 2) are shown in **bold** type. All habitats are ponds except Tera R and Tera D, which are a reservoir and its outflow respectively. The aphally ratio is given with the number of individuals checked for aphally in parentheses. Samples 1, 2 and 3 from 1995 were collected between 11 and 18 January, 1 and 8 February and 20 and 24 February, respectively. T, temporary; S, semi-permanent; P, permanent.

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Table 2. *Population characteristics and aphally ratio in Sardinia and Corsica*

Population	Habitat and localization	Sampling date	Aphally ratio
Corsica			
Orbo	River at the entrance of Ghisonaccia village	11 Sept. 1993	0·91 (71)
Sardinia			
Lotzorai	Stream at the entrance of Lotzorai village	9 Sept. 1993	0·73 (55)
Mare à Bernard	Pond about 7 km north of San Teodoro village	9 Sept. 1993	0·61 (28)

The aphally ratio is given, with the number of individuals checked for aphally in parentheses.

Table 3. *Heterogeneity of the family aphally ratio within natural populations*

Population and sampling	Aphally ratio	No. of families	No. of individuals	Morph homogeneity	Family homogeneity	$t_d \pm$S.E.	$t_c \pm$S.E.
Niger							
NamagaB-3	0·75	23	236	*< 0·0001*	*< 0·0001*	0·37 ± 0·09	0·59 ± 0·14
Taka-1	1·00	13	136	—	*0·02*	0·23 ± 0·10	0·90 ± 0·39
Taka-3	0·98	17	211	*< 0·0001*	*< 0·0001*	0·35 ± 0·10	0·89 ± 0·24
Mari Sud-3	0·71	13	87	*< 0·57*	*0·18*	0·07 ± 0·08	0·17 ± 0·21
Mari Nord-1	0·84	20	157	*< 0·0001*	*< 0·0001*	0·38 ± 0·10	0·67 ± 0·17
Kotaki-1	0·69	48	623	*0·80*	*0·51*	0·00 ± 0·02	0·00 ± 0·03
Bala-1	0·51	20	212	*0·47*	*0·91*	0·00 ± 0·03	0·01 ± 0·05
Bala-3	0·25	24	246	*1·00*	*0·26*	0·05 ± 0·04	0·09 ± 0·08
Niumpalma-2	0·36	23	224	*0·68*	*0·40*	0·03 ± 0·04	0·05 ± 0·06
Niumpalma-3	0·19	12	155	*0·19*	*0·72*	−0·01 ± 0·03	−0·02 ± 0·05
Ligido-1	0·80	26	317	*0·03*	*0·004*	0·16 ± 0·06	0·44 ± 0·15
Doubalma-1	0·74	16	215	*0·11*	*0·24*	0·05 ± 0·04	0·13 ± 0·11
Doubalma-3	0·59	20	202	*0·85*	*0·23*	0·05 ± 0·04	0·12 ± 0·12
Boyze I-1	0·81	20	220	*1·00*	*0·24*	0·05 ± 0·04	0·24 ± 0·21
Corsica							
Orbo	0·91	17	164	*1·00*	*0·02*	0·11 ± 0·07	0·41 ± 0·26
Sardinia							
Lotzorai	0·73	22	309	*0·88*	*< 0·0001*	0·18 ± 0·06	0·36 ± 0·12
Mare à Bernard	0·61	12	223	*1·00*	*0·30*	−0·00 ± 0·02	−0·02 ± 0·06
Data from Doums *et al.* (1996a) on *Niger sampling, 1994*							
Boyze II	0·94	15	229	—	*0·07*	0·06 ± 0·04	0·19 ± 0·15
Namaga B	0·53	77	1039	*< 0·0001*	*< 0·0001*	0·30 ± 0·04	0·48 ± 0·07
Namaga PM	0·69	28	712	*< 0·0001*	*< 0·0001*	0·16 ± 0·04	0·30 ± 0·08
Kobouri	0·00	8	97	—	*0·17*	0·11 ± 0·09	0·89 ± 0·74

The number after each population name refers to the 1995 sampling date indicated in Table 1. The number of families and the number of individuals for which phally state was checked are given for each population studied under laboratory conditions. The probability that the family aphally ratios were similar between the sexual morphs and among families are given in *italics*, as well as the intra-class correlation coefficient estimated on the dimorphic character (t_d) and on the continuous underlying variable (t_c) (see text).

(iii) *Statistical analyses*

In the laboratory study of family aphally ratio among populations, we tested for an effect of population and morph, as well as for their interaction, on the family aphally ratio, using an analysis of deviance which assumes that the error term of the model follows a binomial distribution. After a logit transformation of the data, the model was fitted using a maximum likelihood estimation (McCullagh & Nelder, 1983). To take into account overdispersion, the significance of each term of the model was tested using a *F*-test according to Crawley (1993). This analysis was performed using the software GLIM (Baker & Nelder, 1985).

We tested for heterogeneity of family aphally ratios

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between the two sexual morphs and among families within each population using Fisher's exact test. This gives the probability of observing our pattern of distribution of aphallic individuals among families under the null hypothesis that the offspring of all families originated from the same pool. The probability was calculated using the STRUC program (Raymond & Rousset, 1995). The standard error of these probabilities was always below 0·005. As 40 tests were performed, we applied a Bonferroni correction (Sokal & Rohlf, 1995) (we considered that a test was significant when $P < 0{\cdot}00125$ for a significance level of 0·05). As laboratory conditions can be considered homogeneous, this procedure tests for the occurrence of genetic variability for aphally between the sexual morphs and among families within each population (but see Section 4). We also estimated the proportion of variation in the family aphally ratio that can be explained by family differences (genetic factors). This was done by calculating the intra-class correlation coefficient, giving a value of 1 to aphallic young and 0 to euphallic young and its standard error according to the formula of Hill & Smith (1977). A problem with such an estimate is that the data do not conform to the assumptions of the analysis of variance, since they follow a binomial distribution with a variance dependent on the mean. Hence different expected values of the intra-class correlation would be obtained for a different mean frequency of aphallic individuals (Hill & Smith, 1977). To remove this dependence, a model assuming an underlying continuous variable has often been used (Hazel & West, 1982; Roff, 1986; Falconer, 1989; Doums *et al.* 1996*a*). According to a threshold value of this hypothesized underlying variable, individuals will develop into one morph or the other. Since there are probably several genetic and environmental factors affecting the value of the hypothesized underlying variable, this variable is likely to follow a normal distribution. It is therefore possible to adjust the intra-class correlation calculated on the discontinuous scale (r_d) (binary data) to the frequency-independent correlation on the continuous scale (r_c) (underlying variable) (Hill & Smith, 1977). As the offspring are probably genetically very similar, the intra-class correlation coefficients can be considered as the broad sense heritability (Falconer, 1989). However, this calculation must be taken cautiously since we have no direct evidence from our data for the validity of the threshold model. We therefore present the values of the two intra-class correlation coefficients. Analyses on the family aphally ratio were performed only on families with at least five offspring.

The aphally ratio of natural populations was compared with that observed among the offspring of all individuals from all samples. The offspring aphally ratio of the population was calculated as the mean of the offspring aphally ratio of the aphallic and euphallic individuals weighted by the aphally ratio of the natural population at the sampling date. We calculated the Pearson product–moment correlation coefficient between the two values after arcsine transformation of the aphally ratio. For the populations sampled twice, each sampling was taken into account since change in the population aphally ratio as well as in the offspring aphally ratio could occur between the two sampling dates. We also tested for an effect of water availability on the aphally ratio and on the intra-class correlation coefficient by contrasting populations occupying relatively stable (semi-permanent and permanent) and temporary environments, using populations from Niger only for which water permanence of the habitat is known. The effect was tested using a Kruskal–Wallis one-way analysis of variance (Sokal & Rohlf, 1995, p. 424). For the populations sampled twice, we used the mean value of the parameters. For all these comparisons among populations, we took into account data on the offspring aphally ratio for four populations from Niger, studied in 1994 by Doums *et al.* (1996*a*), to obtain a larger data set (see Table 3).

3. Results

(i) *Variability of the aphally ratio among populations*

The aphally ratio was significantly heterogeneous among populations whatever the sampling data (Table 1; Fisher exact test, $P < 0{\cdot}0001$ for all sampling dates). The population aphally ratio between sampling dates was significantly correlated within one year as well as between the two years of sampling (Fig. 2). In the laboratory, we observed a significant population effect on the offspring aphally ratio (Table 4). Our results also indicated a significant correlation between the aphally ratio estimated in natural populations and the mean population aphally ratio among G_1 offspring (Fig. 3). The coefficient of determination of this correlation was 0·67, indicating that 67% of the difference in aphally ratio among natural populations can be explained by differences in the family mean aphally ratio observed under laboratory conditions among the populations.

All populations exhibiting an aphally ratio lower than 50% are situated in a geographically restricted area (populations 10, 11, 12, 13 and 14 in Fig. 1). However, no correlation was observed between the difference in aphally ratios between pairs of populations and the linear geographic distance (log transformed) separating them (Mantel test, 100000 permutations; 1994: $P = 0{\cdot}67$, mean aphally in 1995: $P = 0{\cdot}79$).

(ii) *Variability of the family aphally ratio within populations*

The family aphally ratio was highly variable and its distribution was continuous when families from all

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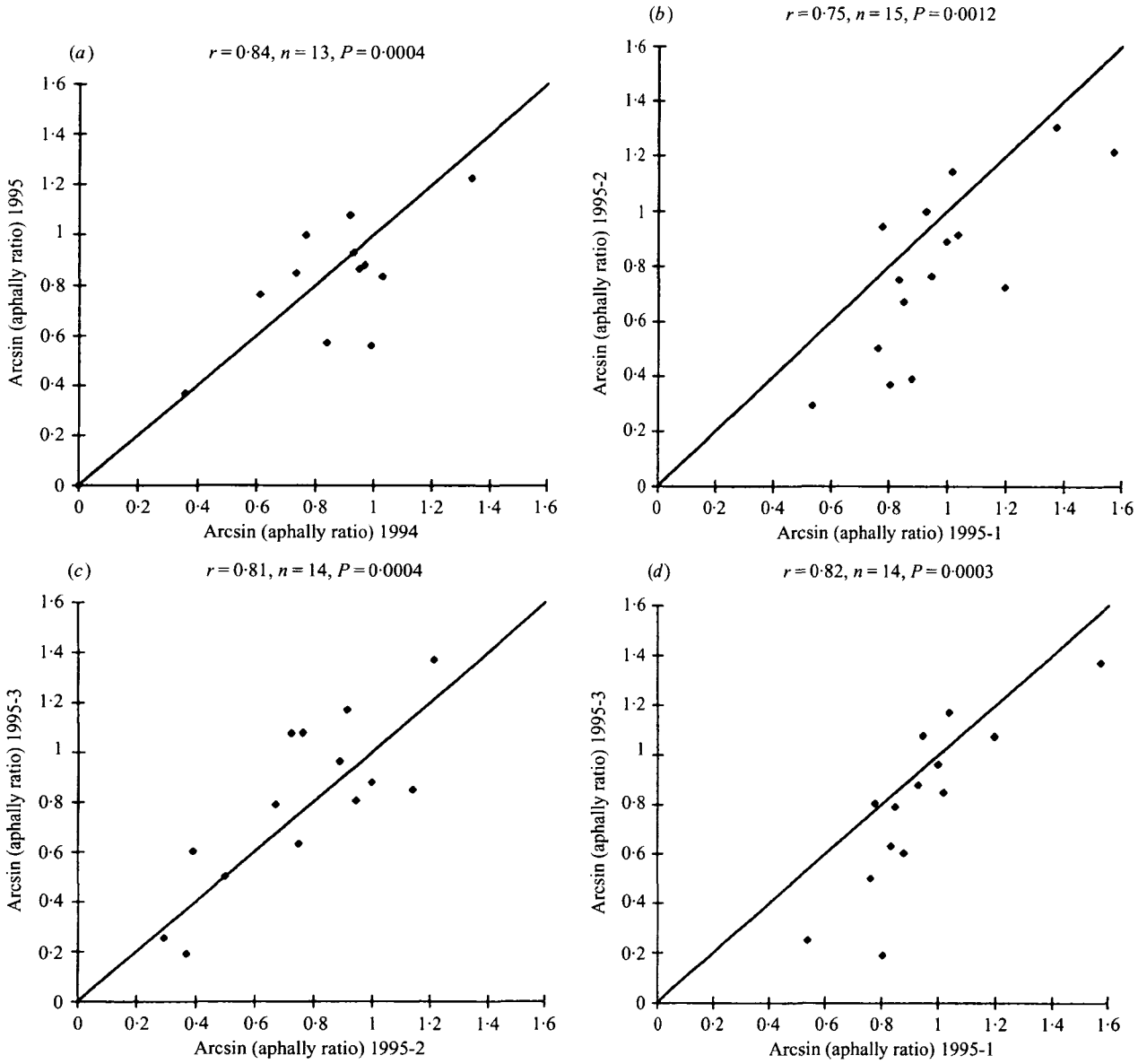

Fig. 2. Relationship in natural populations between the aphally ratios of different sampling dates. (*a*) Refers to the comparison between 1994 and the mean aphally ratio in 1995; (*b*) between the sampling dates 1 and 2; (*c*) between the sampling dates 2 and 3, (*d*) between the sampling dates 1 and 3 in 1995 (as given in Table 1). Each point is a population. The continuous line is the 1:1 ratio.

Table 4. *Results of the analyses of deviance testing the population and morph effects and their interaction on the family aphally ratio*

Factors	Scaled deviance	Degrees of freedom	*F*-test	*P* value
Population	332·69	12	13·05	< 0·0001
Morph	16·56	1	7·79	0·006
Population × Morph	86·07	12	3·38	0·0001
Error	679·845	320		

Details of the analyses are given in the text.

populations were plotted together (Fig. 4). It also appeared that aphallic individuals produced on average more aphallic offspring than did euphallic individuals, as shown by the position of the curves in Fig. 4. This effect of parental morph on the family aphally ratio was significant (Table 4). These observations suggest that a large number of factors are involved in the determination of aphally and that we can assume a polygenic basis to aphally. Moreover, we observed a significant interaction term between the

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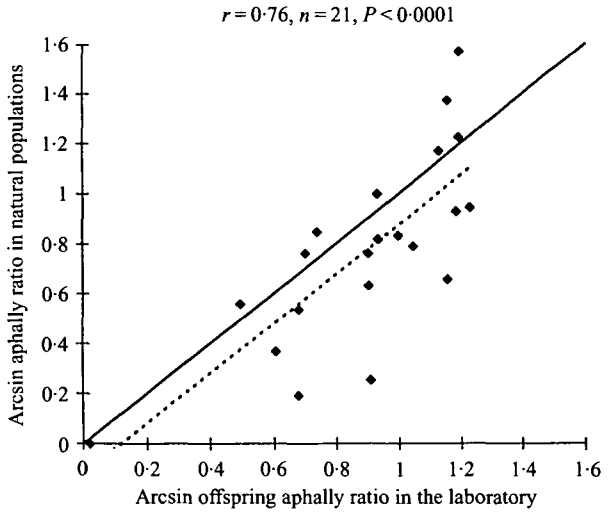

Fig. 3. Relationship between the aphally ratio of natural populations and the aphally ratio observed among the G_1 offspring under laboratory conditions. Each square is a population sampling (Table 3). The continuous line is the 1:1 ratio and the dashed line is the regression line.

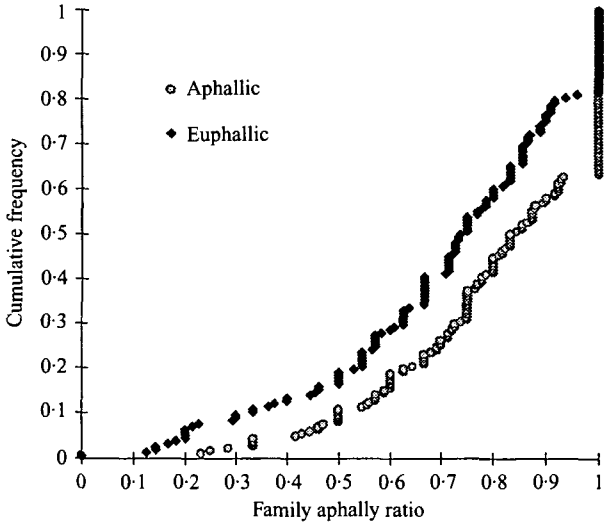

Fig. 4. Cumulative frequency of the family aphally ratio within the two sexual morphs. Each point is an individual characterized by its family aphally ratio and the frequency of individuals with lower family aphally ratios. This curve has been plotted using all families studied (346).

morph and population effects on the family aphally ratio (Table 4). This is due to the fact that aphallic individuals produced significantly more aphallic offspring than did euphallic individuals only for populations having a significant heterogeneity for the family aphally ratio (Table 3). However, one population (Lotzorai) did not conform to this rule.

Four populations out of 13 presented a significant heterogeneity for the family aphally ratio (Table 3). High values of the intra-class correlation coefficients (notice the low S.E.S) were associated with significant heterogeneities. There was a positive significant correlation between the estimates of the correlation coefficient calculated on the dimorphic and continuous scale (Spearman rank correlation, $r_S = 0{\cdot}92$; $n = 21$; $P < 0{\cdot}0001$). The difference between the two coefficients occurred when the probability of being aphallic is near zero or unity, as expected following Dempster & Lerner (1950). The intra-class correlation coefficient was not correlated with the number of families studied in each sampling (Spearman rank correlation, dimorphic scale: $r_S = 0{\cdot}19$, $n = 21$, $P = 0{\cdot}40$; continuous scale: $r_S = 0{\cdot}00$, $n = 21$, $P = 1{\cdot}00$). These results suggest that the low intra-class correlation observed in some populations was due to the absence of genetic variability rather than to a higher total phenotypic variation for phally polymorphism. Therefore, in most of the populations, there is no genetic variability for aphally on which selection could act. The results for the five populations studied more than once were similar within and between years (Table 3).

There was no significant correlation between the mean intra-class correlation coefficients and the mean aphally ratio of the populations (Spearman rank correlation, dimorphic scale: $r_S = 0{\cdot}43$, $n = 16$, $P = 0{\cdot}10$; continuous scale: $r_S = 0{\cdot}40$, $n = 16$, $P = 0{\cdot}12$). This is easily explained since populations with intermediate of high aphally ratio can be variable or invariable (example of Taka and Orbo for populations with high aphally ratio and Namaga B and Doubalma; see Table 3). The presence or absence of variability in populations with intermediate aphally ratio means that, in these populations, individuals either all have an intermediate family aphally ratio or exhibit some variability which averages over the population to an intermediate aphally ratio.

No effect of water availability was observed, either for the population mean aphally ratio ($H = 0{\cdot}24$, $n = 13$, $P = 0{\cdot}62$) or for the mean intra-class correlation coefficient (dimorphic character: $H = 2{\cdot}06$, $n = 13$, $P = 0{\cdot}15$; continuous character: $H = 1{\cdot}53$, $n = 13$, $P = 0{\cdot}22$).

4. Discussion

Our results show that the aphally ratio is variable among natural populations and that this variation is apparently stable over time. They also indicate that phally polymorphism is largely determined by genetic factors. Our study is also the first large investigation of the within-population variability for aphally. Four populations out of 13 presented some genetic variability for aphally. An interesting result was that the within-population variability for aphally can be observed in populations expressing a wide range of aphally ratios. For example, populations with intermediate aphally ratio may or may not present some genetic variability. No effect of water availability in natural habitats was detected for either the aphally ratio or the intra-class correlation coefficients of the populations.

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(i) *Between-population variability*

The high variability of the aphally ratio observed here among natural populations from Niger was previously observed by Schrag *et al.* (1994*b*) in a survey of Nigerian populations of *B. truncatus*. In their survey, a positive correlation between aphally ratios measured at an interval of 2 months in natural populations has also been found ($r = 0{\cdot}69$, $n = 20$, $P < 0{\cdot}001$). The difference in aphally ratio between populations was relatively stable, just as in our study, though the aphally ratio tended to decrease over the course of the study. Schrag *et al.* (1994*b*) related this phenomenon to the decrease in maximum air temperature 5 weeks prior to snail collection, which corresponded approximately to the temperature during phally development (Schrag & Read, 1992). An effect of temperature on the aphally ratio has also been observed in laboratory experiments (Schrag & Read, 1992). Other environmental factors may also influence phally determination in the field and could be responsible for the difference in aphally ratio observed among populations. Although environmental factors play a role, we showed that 67% of the difference in aphally ratio between populations is due to genetic differences. This provides the strong genetic basis required for further studies of the selective forces shaping the distribution of phally polymorphism in the light of the theory of natural selection.

(ii) *Within-population variability*

The high variability observed for the family aphally ratio across all sampled individuals requires that many factors determine the sexual morph. Some of these factors could be environmental, since all offspring from a given parent were reared in the same box. However, environmental variation among boxes was very low under laboratory conditions. Moreover, a previous study conducted in the laboratory on three populations has shown that the family aphally ratio is stable from one generation to the next (Doums *et al.* 1996*a*). This suggests that the differences among individuals in the family aphally ratio have a genetic basis. It is not possible, from our data, to determine whether the genetic factors involved in phally polymorphism are uniparentally or biparentally inherited. More insight would be gained through crossing experiments. However, it is difficult to obtain outcrossed progenies using snails from highly selfing populations (Jarne *et al.* 1993). The few crosses successfully performed by Larambergue (1939), using lineages differing in their family aphally ratio, showed that the offspring aphally ratio of an individual changed when switching from selfing to outcrossing, indicating a paternal effect on offspring aphally ratio. More data are required to confirm these preliminary results.

The threshold model, involving a continuous underlying variable (see Section 2), may be used to explain the determination of aphally, assuming that many factors determine the sexual morph. However, the value of the intra-class correlation coefficient must be interpreted cautiously and can only be viewed as an indication of the magnitude of genetic variability (among families) determining the sexual morph. It gives the ratio of the total phenotypic variation explained by genetic variation. It therefore indicates whether some genetic variability is present on which selection could act, although it cannot be interpreted as narrow sense heritability (Falconer, 1989). The intra-class correlation coefficient was not correlated with the number of families studied, and therefore, does not appear strongly biased by this potential effect. The aphally ratio of the population is not representative of the genetic variability. Intermediate population aphally ratios may be observed even if there is no variability for the family aphally ratio. In such populations, individuals whatever their sexual morphs produced the same offspring aphally ratio. This can be explained in the context of the threshold model by adding a stochastic process to explain that two genetically similar individuals can have different sexual morphs, even under homogeneous environmental conditions (Doums *et al.* 1996*a*). Over all populations, including those of Doums *et al.* (1996*a*), the genetic variability for aphally within populations was observed for five populations out of 16 (see Table 3).

The high level of genetic variation for aphally observed in some populations is somewhat surprising, given the absence of electrophoretic variability within populations in this species (Jelnes, 1986; Njiokou *et al.* 1993). However, substantial within-population variability has been observed using microsatellite markers, indicating that high mutation rates, such as those of microsatellites, can maintain some within-population genetic variability (Jarne *et al.* 1994; Viard *et al.* 1996).

(iii) *Genetic variability of the aphally ratio: selected or neutral pattern*?

Within-population variability differs greatly among populations. What then determines the distribution of genetic variability among natural populations? Both selective factors, acting via selection on the selfing rate or directly on aphally, and stochastic factors, especially recurrent population bottlenecks, may be involved.

Three major selective factors can act differentially on the sexual morphs through selection on the selfing rate, assuming a positive correlation between the family aphally ratio of a lineage and its mean selfing rate. First, high inbreeding depression, which corresponds to a lowered fitness of selfed offspring when compared with outcrossed offspring (Charlesworth & Charlesworth, 1987), may limit any increase in the

selfing rate and therefore in aphally. As inbreeding depression is not a static parameter but may vary with the inbreeding history of the populations (for review see Uyenoyama *et al.* 1993; Jarne & Charlesworth, 1993), it may vary among populations and individuals and be the basis for differential selection among populations. We showed elsewhere that inbreeding depression is limited in two of the populations studied here (Mari and Kobouri: Doums *et al.* 1996*b*). Secondly, selection for outcrossing has been predicted to vary with the ability to find a mate (Schmitt & Ehrhardt, 1987; Jarne *et al.* 1993). This hypothesis, known as the reproductive assurance hypothesis (Stebbins, 1957), predicts that selfing is more common when the opportunity to find a mate is reduced. In other words, selfing would be more common when density is low. In the environment we sampled, estimating density is not easy since (i) our density estimates may vary daily with variations in local conditions (e.g. wind) and (ii) density itself depends on availability of supports (pieces of wood, water lily). Overall density estimates based on limited sampling in time and space are poor indicators of the opportunity to find a mate. Perhaps due to these difficulties, Schrag *et al.* (1994*a*) failed to observe any correlation between density and aphally ratio in natural populations. Third, ecological factors based on the benefits of producing variability offspring when the environment is variable, may also be involved in the evolution of selfing and phally polymorphism (Jarne *et al.* 1993; Schrag *et al.* 1994*a*). One major selective pressure to maintain the production of variable offspring is the action of parasites, a hypothesis that has come to be known as the Red Queen hypothesis (Bell, 1982). This hypothesis assumes that coevolution between parasites and hosts can select for the rarer host genotype through frequency-dependent selection, and therefore select for increased recombination rate among hosts (review in Hamilton *et al.* 1990). The potential effects of parasites on the evolution of the selfing rate have rarely been addressed (but see Lively & Howard, 1994). As *B. truncatus* is a vector for numerous parasitic trematodes, parasitism may act as a selective factor to maintain euphallic individuals through selection for outcrossing. Schrag *et al.* (1994*a*) indeed observed a significant negative correlation between the aphally ratio and the prevalence of one group of trematode parasites.

As mentioned above, these factors can influence the distribution of aphally in natural populations only if there is a positive relationship between the selfing rate and the aphally ratio. However, recent studies performed in *B. truncatus* have shown that euphallic individuals originating from two Niger populations mainly self-fertilize under laboratory conditions even when a partner is available. This study is based on both estimates of inbreeding depression and genetic analysis of crosses using microsatellite markers (Doums *et al.* 1996*b*). In natural populations, genetic studies failed to show any correlation between the inferred selfing rate and the aphally ratio, with all populations estimated to self-fertilize at a rate of 0·8–1, whatever their aphally ratio (Viard *et al.* 1996). These high values are confirmed by results from progeny-array analyses conducted in five of the populations studied here (F. Viard & P. Jarne, unpublished). We therefore have independent evidence of high selfing rates whatever the aphally ratio. Clearly, variation in the selfing rate is limited, and we can wonder whether such a limited variation allows any influence of the selective factors cited above on phally polymorphism in natural populations.

Alternatively, phally polymorphism may be an almost neutral character with regard to the selfing rate. The evolution of high aphally ratios would be driven by differences in life-history traits between the two sexual morphs. It may indeed be that the phallus of euphallic individuals represent a cost, and that the energy saved by aphallic individuals is re-allocated to another function. This has been investigated in a series of experiments in which growth, survival and reproduction were followed under various laboratory conditions. The only observed differential bears on the production of eggs, which is lower in euphallic than in aphallic snails. This has been interpreted as revealing the cost of the phallus (Jarne *et al.* 1992; Schrag & Rollinson, 1994). However, more recent work taking into account possible bias such as small sample size and family-correlated reproductive output failed to show such a difference (Doums & Jarne, 1996*a*), even under stressful conditions (Doums & Jarne, 1996*b*; but see Schrag & Rollinson, 1994).

The distribution of the genetic variability for aphally is probably also determined by the inbreeding history of natural populations. Indeed, high selfing rates and genetic bottlenecks affect the distribution of the neutral genetic variability within and between populations (Nei *et al.* 1975; Charlesworth *et al.* 1993). Genetic analyses using microsatellite data effectively point to the strong role of population bottlenecks in the distribution of neutral genetic variability (Viard *et al.* 1996). We did not observe an effect of water availability in natural habitats on either the population aphally ratio or the intra-class correlation coefficients. However, the number of populations studied was quite limited and water availability is only an indirect indicator of the magnitude of population bottlenecks. Even in permanent ponds, population density may be dramatically reduced during the dry season (Vera *et al.* 1995). Genetic drift following population bottlenecks may therefore occur whatever the habitat. A comparison of genetic variability observed using neutral markers, particularly microsatellite DNA, with that observed for aphally could indicate the role of genetic drift in the distribution of genetic variability for aphally. We note first that there is no relationship between the aphally ratio in natural populations and microsatellite variability (Viard *et al.* 1996). This is

consistent with the absence of correlation between the selfing rate and the aphally ratio. On the other hand, based on eight populations for which the variability for microsatellites (Viard *et al.* 1996) and for aphally was known, all populations with a relatively high mean number of alleles per locus also expressed with significant variability for aphally. This suggests that even if aphally is a selected trait, population dynamics is a major force shaping the distribution among populations of genetic variability for aphally.

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