The Genetical and Environmental Determination of Phally Polymorphism in the Freshwater Snail *Bulinus truncatus*

Claudie Doums,* Philippe Bremond,† Bernard Delay* and Philippe Jarne*

**Institut des Sciences de l'Evolution, Université Montpellier II, Montpellier, France and †C.E.R.M.E.S., Niamey, Niger*

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ABSTRACT

In some species of self-fertile pulmonate snails, two sexual morphs co-occur in natural populations: regular individuals and aphallic individuals that cannot transmit sperm to other snails. Purely aphallic populations therefore reproduce obligatorily by selfing. Understanding the evolution of aphally and selfing in these snails requires a precise knowledge of phally determination. In this paper, we investigate the genetic and environmental determination of aphally in *Bulinus truncatus* by a survey of the family (offspring) aphally ratio of 233 individuals originating from seven natural populations and a study of the reaction norm of the family aphally ratio to temperature using 60 individuals from 10 selfed lineages of one population. Our results indicate a high genetic variability for the determination of aphally between populations and within some populations, associated with a high level of genetic determination. Our second experiment indicates a significant temperature and lineage effect though no interaction between these two effects. We discuss our results in the framework of threshold models developed for dimorphic traits with polygenic inheritance. We propose that the sexual morph of an individual at a given temperature is determined by a temperature threshold value depending on both the individual genotype and probabilistic processes.

SEXUAL polymorphisms, that is, the co-occurrence of sexual morphs within the same population (DARWIN 1877), provide the opportunity to study the forces driving the evolution of self-fertilization *vs.* cross-fertilization through the variation of the ratio of the sexual morphs. Indeed this ratio directly influences the selfing rate in the population. For instance, an increase in the ratio of male-sterile over hermaphroditic individuals in gynodioecious plant species decreases the selfing rate in a population since male-sterile individuals cannot reproduce by selfing (GOUYON and COUVET 1987). Alternatively, an increase in the ratio of cleistogamous over chasmogamous flowers in cleistogamous plant species increases the selfing rate in the population since cleistogamous flowers obligately reproduce by selfing (SCHOEN and LLOYD 1984). In such sexually polymorphic species, a high variability of the ratio of the sexual morphs is generally observed (*e.g.*, GOUYON and COUVET 1987), a favorable condition for studying the factors driving the evolution of sexual polymorphisms. However, sexual polymorphisms are not restricted to plants, as shown by some self-fertile hermaphroditic snail species (Pulmonata, Gastropoda). These species are characterized by the occurrence of regular hermaphroditic individuals, referred to as euphallic individuals, together with aphallic individuals, which are deprived of the male copulatory organ but can still self-fertilize (LARAMBERGUE 1939). Such a dimorphism has a direct influence on the selfing rate since selfing is obligatory in strictly aphallic populations. Moreover, the aphally ratio (frequency of aphallic individuals) is highly variable in natural populations (LARAMBERGUE 1939; BAUR and CHEN 1993; SCHRAG *et al.* 1994a), which allows investigation of the ecological factors driving this variation and that of the selfing rate (JARNE *et al.* 1992; SCHRAG *et al.* 1994a). A fundamental assumption of any model explaining the evolution of sexual dimorphisms is that the sexual morphs are, at least partly, genetically determined. Although SCHRAG *et al.* (1992) failed to show any heritability of aphally using a selection experiment and a sib-family analysis in the freshwater snail *Bulinus truncatus*, results of LARAMBERGUE (1939) indicated some genetic determination in the same species. LARAMBERGUE (1939) obtained selfing lineages characterized by a very high or a very low offspring aphally ratio whatever the sexual morph of the parent. It has also been shown that phally polymorphism is determined by environmental factors both in laboratory conditions and in natural populations: the aphally ratio increases with an increase of the rearing temperature of eggs (SCHRAG and READ 1992). This effect of temperature on the determination of the sexual morph has been interpreted as an adaptive characteristic (SCHRAG and READ 1992; SCHRAG *et al.* 1994a) paralleling arguments developed for environmental sex determination (CHARNOV and BULL 1977). However, this interpretation requires a more detailed knowledge of the respec-

Corresponding author: C. Doums, Génétique et Environnement CC065, Institut des Sciences de l'Evolution, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier cedex 5, France.
E-mail: doums@isem.univ-montp2.fr

tive roles of genetic and environmental factors, as well as of their interaction, in the determination of aphally.

In this paper, we investigate both the level of genetic determination of aphally in many populations and the interaction between genetic and environmental factors. The species studied is the hermaphrodite freshwater snail *B. truncatus* (Gastropoda, Planorbidae), the most widely studied species in relation with aphally. This snail is one of the intermediate African hosts of the human-infecting trematode Schistosoma (BROWN 1994). *B. truncatus* is an allotetraploid species with a distribution from the South of Africa to the Middle East and the Mediterranean Islands (BROWN 1994). This species has also been intensely studied for its allozyme and DNA polymorphisms, its mating system and population dynamics (review in BROWN 1994). A reproduction by selfing with limited or no inbreeding depression (JARNE *et al.* 1992) and a short generation time make this species particularly convenient for family analysis of the aphally ratio since large numbers of selfed offspring can be obtained from single individuals. We first analyze the level of genetic determination considering aphally as a threshold character (ROBERTSON and LERNER 1949; DEMPSTER and LERNER 1950; FALCONER 1989, chapter 18) through sib-family and parent-offspring regression at constant temperature. We assume that the development of the phallus is determined by the level of some undetermined variate such as a hormonal factor. The phenotypic expression of the dimorphic character is determined by the mean of liability of this underlying variate for which a threshold value switches the phenotype from one morph to the other. This mean liability is assumed to be dependent on many genetic and environmental factors that distribute it on a continuous normal distribution as a quantitative character. Second, we analyze the response to temperature of 10 lineages originating from one population by following the family aphally ratio. The variation of the mean family aphally ratio of the lineages with the variation in temperature is analyzed as a reaction norm. An individual can be characterized by its sensitivity to temperature, which corresponds to the slope of the regression lines of the family aphally ratio on temperature, and its mean performance, which is the mean of its family aphally ratio over the three temperatures (FALCONER 1990). The variability of the family aphally ratio at a given temperature between individuals may be due to differences in sensitivity and/or mean performance. Differences in sensitivity among families would indicate an interaction between genetic and environmental factors.

MATERIALS AND METHODS

Rearing conditions: Individuals were reared individually in 80-ml plastic boxes at 25° with a 12/12 photoperiod. They were provided *ad libitum* with boiled lettuce. Water and food were changed three times a week. Temperature determines the sexual morph during egg development and sometimes during the first days after hatching (SCHRAG and READ 1992). The rearing temperature of parents has no effect (SCHRAG and READ 1992). In the reaction norm experiment, the parents were kept at 25° and eggs were collected every day and placed at the temperature analyzed (eggs were at most 24 hr old). Young snails were maintained at the tested temperature up to the determination of the sexual morph. The sexual morph can easily be identified for individuals larger than 3 mm by observation of narcotized individuals under a binocular microscope.

The genetic determination of aphally: The family aphally ratio, defined as the frequency of aphallic individuals among offspring, was estimated at a constant temperature of 25° for 233 individuals originating from six natural populations (Table 1). Namaga PM and Namaga B are separated by only a few hundred meters but were analyzed separately because of different aphally ratio. For each population, the G_0 individuals (generation 0 individuals originating from natural populations) were isolated in the laboratory, and their eggs (G_1 = generation 1) were collected over a few days, giving a mean number of 16.8 offspring per individual (SE = 14.22, min = 5, max = 67) that were checked for their sexual morph. The ratio of selfed G_1 offspring was unknown. Indeed, snails can store the sperm of copulation partners for some time and use it when subsequently isolated (JARNE *et al.* 1993). Individuals from Mari were kept isolated over 2 months in the laboratory before egg collection, a period long enough to ensure the exhaustion of foreign sperm and therefore self-fertilization (JARNE *et al.* 1993). However, for the other populations, G_0 individuals were kept isolated in the laboratory only a few days after field sampling. The large heterozygote deficiencies observed with allozyme (NJIOKOU *et al.* 1993) and microsatellite (JARNE *et al.* 1994) markers, the limited inbreeding depression (JARNE *et al.* 1992; C. DOUMS, unpublished data) and the limited propensity to copulate point to a high selfing rate in natural populations of *B. truncatus*.

For the populations of Mari, Namaga PM and Namaga B, we also followed the ratio of aphallic offspring for some individuals over two generations to test for the stability of the offspring aphally ratio. The second generation was engendered through selfing since individuals were isolated before sexual maturity. More than one G_1 offspring for each G_0 individual were used in some cases. We then calculated the aphally ratio using all offspring of the sibling G_1 individuals.

In the populations of Mari, Dyoro, Namaga PM and Namaga B, where both aphallic and euphallic G_0 individuals occurred, we compared the aphally ratio of the offspring from the two morphs using Fisher's exact tests. Genetic determination would be indicated by a higher ratio of aphallic offspring among aphallic individuals. We also estimated the level of genetic determination or broad sense heritability (FALCONER 1989, chapter 10) for each of these populations using both the intraclass correlation coefficient obtained from G_1 sib-family analyses and parent-offspring regressions (FALCONER 1989, chapter 10). The direct application of these quantitative genetics methods (primarily derived for normally distributed traits) to dimorphic characters following a binomial distribution is problematic, partly because the binomial variance depends on the mean, violating a basic assumption of classical analyses of variance. We therefore also analyzed the level of genetic determination considering aphally as a threshold character (see Introduction). For the sib-family analysis, the level of genetic determination is given by the intraclass correlation coefficient, which was computed, as well as its standard deviation, both directly from the dimorphic character and for the underlying variate, according to HILL and SMITH (1977). The intraclass correlation coefficient of the underlying variate was also estimated using the probands methods (FALCONER

TABLE 1

Population characteristics

Population	Location	Type	Aphally ratio
Beni-Abbès	Algeria (4°W; 30°N)	Dam	0.98 (112)
Mari	Niger (1°E; 14°N)	Semipermanent pond	0.78 (74)
Dyoro	Burkina faso (2°W; 12°N)	Dam	0.43 (379)
Boyze II	Niger (4°E; 13°N)	Permanent pond	0.94 (165)
Kobouri	Niger (1°E; 12°N)	Permanent pond	0.00 (23)
Namaga PM	Niger (1°E, 14°N)	Semipermanent pond	0.69 (64)
Namage B	Niger (1°E, 14°N)	Semipermanent pond	0.53 (192)

The aphally ratio is provided with the number of individuals checked for their sexual morph in parentheses.

1989, chapter 18). Aphallic individuals were assumed to be independent "events" and treated as probands. The intraclass correlation of the continuous variate can be estimated directly from the frequency of probands in the population and among relatives, this latter being estimated from the frequency of aphallic individuals in each family according to HILL and SMITH (1977). In the parent-offspring regression, the level of genetic determination is given by the slope of the regression. The regression was applied after the family mean aphally ratio was arcsine transformed. We also estimated the slope of the parent-offspring regression for the underlying variate by transforming the ratio of aphallic offspring in each family in the scale of the underlying variate (ROFF 1986a). This was performed by taking the value of the abscissa on the standardized normal curve corresponding to the proportion of aphallic offspring in the family (ROFF 1986a). The level of genetic determination was directly assessed from the intraclass correlation and the slope of the parent-offspring regression, since we assumed that all offspring were selfed and that therefore the coefficient of relatedness was close to one. However, as mentioned above, some of the G_1 snails were probably produced by outcrossing that induced an underestimation of the level of genetic determination.

Reaction norms to temperature: The sensitivity to temperature was investigated using the offspring of 60 G_3 parental snails originating from 10 lineages of the population of Mari. These lineages differed by their family aphally ratio and were characterized by a stable family aphally ratio over three generations of selfing. In each generation, one individual was used to engender the following generation. For the five lineages characterized by an intermediate family aphally ratio at 25°, four aphallic and four euphallic sibling individuals (G_3) were used to constitute the tested generation. For the three lineages with a low family aphally ratio, only four sibling euphallics were used, and for the two last lineages with a high family aphally ratio, four sibling aphallics were used. Three water temperatures were tested: low (19°), intermediate (25°) and high (31°). A schematic diagram of the breeding experiment is given in Figure 1. The variation of water temperature did not exceed 1°. For each G_3 parent reared at 25°, egg capsules were collected until at least 20 eggs were obtained, that is, over 2 or 3 days. This was successively performed at 19°, 25° and 31°. The number of eggs collected at each temperature tested was also recorded to estimate the hatching rate and survival of young up to the determination of the sexual morph (~4 weeks).

The interaction between genotypic and environmental factors was assessed through an analysis of deviance with temperature and lineage factors as the factorial main effects, and morph and parent effects nested under lineage and morph effects, respectively. The probability of being aphallic can be expressed as

$$p_{ijkl} = u + t_i + g_j + m_{jk} + r_{jkl} + tg_{ij} + tm_{ijk} + e_{ijkl},$$

where p_{ijkl} is the probability of being aphallic for the offspring of parent l with sexual morph k within lineage j in the environment (temperature) i, u is the mean probability of being aphallic for all individuals, t_i is the deviation to the mean due to temperature i, g_j is the deviation due to lineage j, m_{jk} is the deviation due to sexual morph k in lineage j, r_{jkl} is the deviation due to parent l with sexual morph k of lineage j and e_{ijkl} is the error term. We assumed that the error term follows a binomial distribution and, after a logit transformation of the data, the model was fitted using a maximum likelihood estimation (MCCULLAGH and NELDER 1983). First we fit the full model, then we remove, one after the other, the nested and nonsignificant terms. To account for overdispersion, significance testing of each term of the model proceeds by calculating the ratio of the change in scaled deviance resulting from the removal of a term of the current model over the scaled deviance of the current model weighing by their associated degrees of freedom. This ratio is distributed as F. For each term tested, the current model is characterized by the terms included in its deviance (see Table 4). Only individuals for which we obtained offspring at the three temperatures ($N = 43$) were considered

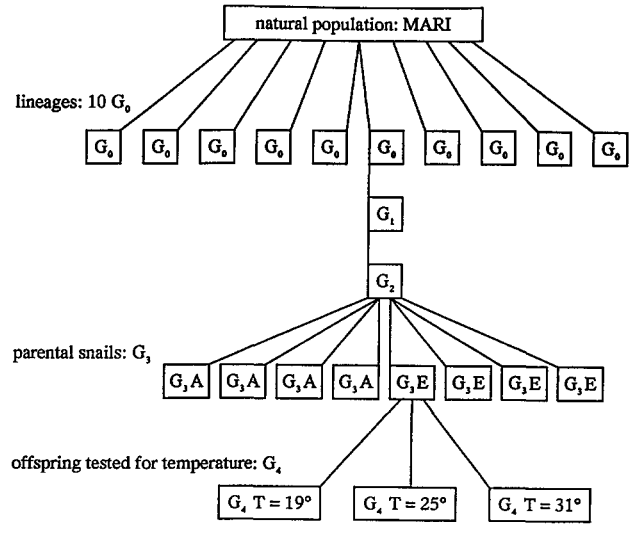

FIGURE 1.—Breeding design for obtaining the parental snails of the reaction norm experiment. The breeding design depicted here for one G_0 lineage and one parental G_3 snail is the same for all the 10 G_0 and 60 G_3 parental snails in the overall experiment (see text). Each square represents one individual and all generations are obtained under selfing. A, aphallic; E, euphallic.

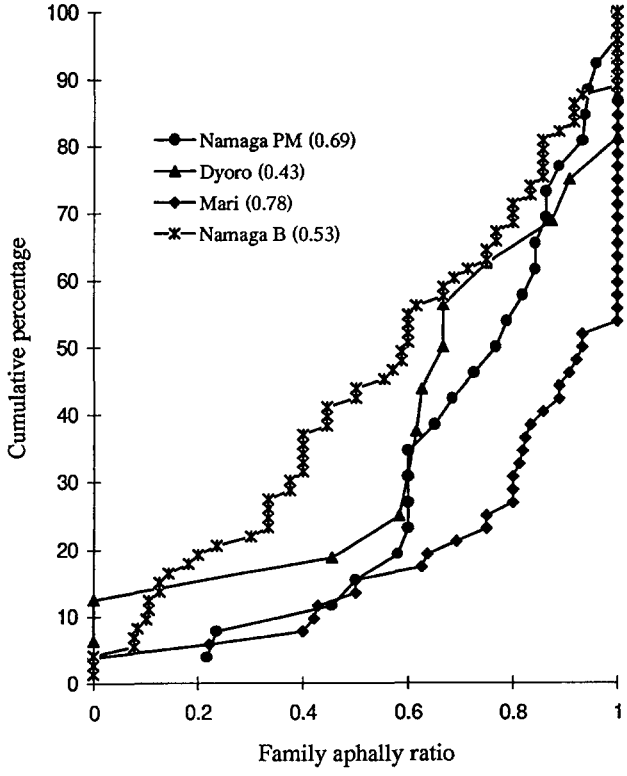

FIGURE 2.—Cumulative frequency of the family aphally ratio within populations. Each point is an individual characterized by its family aphally ratio and by the percentage of individuals with lower family aphally ratios. The family aphally ratio was estimated at a constant temperature of 25° for 175 individuals originating from four natural populations. The aphally ratio in the natural population is given in parentheses for each population.

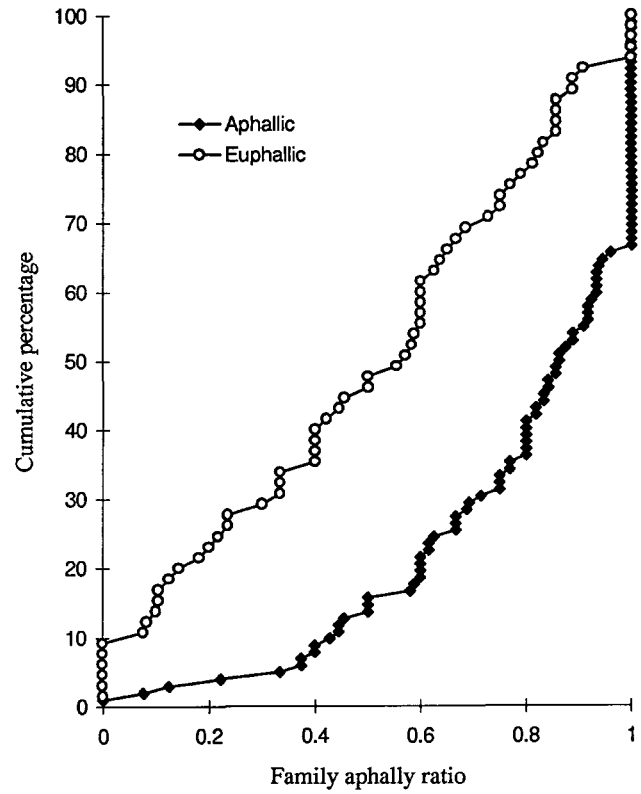

FIGURE 3.—Cumulative frequency of the family aphally ratio within sexual morphs. Data are as in Figure 2.

in this analysis to avoid missing data. Note that we obtained similar results using the full data set. Survival was analyzed in the same way, p_{ijkl} being the probability of being alive at phally determination (including hatching rate and survival of young) for an offspring of parent l with morph k within lineage j at temperature i. All calculations were carried out using the package GLIM (BAKER and NELDER 1985).

RESULTS

The genetic determination of aphally: Individuals originating from the populations with extreme aphally ratio (AR) (Kobouri, AR = 0%; Beni Abbès, AR = 100%; and Boyze, AR = 94%) were characterized by a family aphally ratio similar to that of their population. The nine individuals from Kobouri produced a family mean aphally ratio of 0.02 (min = 0.00, max = 0.17), the 17 individuals from Boyze, a mean of 0.9 (min = 0.8, max = 1) and the 32 individuals from Beni-Abbès, a mean of 0.99 (min = 0.95, max = 1.00). On the other hand, individuals from populations with an intermediate aphally ratio exhibited various family aphally ratios (Figure 2). The cumulative frequency distribution of the family aphally ratios differed between populations. For example, ~50% of the individuals from Mari produced only aphallic offspring against only ~10% for Namaga B (Figure 2). The comparison of these curves between aphallic and euphallic individuals merging data from these four populations (Figure 3) indicated that the two sexual morphs could produce any aphally ratio among their offspring. However, that the curve of aphallic individuals was below that of euphallic individuals indicated that there was a larger number of families producing a high aphally ratio among the aphallic individuals. Indeed for these populations aphallic individuals produced a significantly higher number of aphallic offspring than euphallic individuals (Table 2).

The broad sense heritability was estimated from the four populations showing some variability for the family aphally ratio using both the intraclass correlation coefficient and the parent-offspring regression (Table 3). The intraclass correlation coefficient was always higher than 0, indicating that offspring were more similar within than among families. Moreover, the slope of the parent-offspring regression on the family aphally ratio was always significantly different from 0. The intraclass correlation was even higher when estimated for the underlying continuous variate. However, its relative magnitude for each population was identical using both methods. The Mari and Dyoro populations showed the highest levels of genetic determination, which may in part be due to a higher rate of selfed offspring in these populations, especially Mari (see MATERIALS AND METHODS). Our results indi-

TABLE 2

Comparison of the aphally ratio among the offspring of aphallic and euphallic individuals

Population (aphally ratio)	Na	ARa	Ne	ARe	*P*
Mari (0.78)	42	0.85 (470)	12	0.74 (144)	<0.00001
Namaga PM (0.69)	14	0.86 (374)	14	0.62 (338)	<0.00001
Namaga B (0.53)	37	0.64 (497)	40	0.29 (542)	<0.00001
Dyoro (0.43)	9	0.81 (118)	7	0.48 (52)	<0.00001

Na and Ne are the number of aphallic and euphallic G_0 individuals analyzed, respectively. ARa and ARe are the aphally ratios among the G_1 offspring of G_0 aphallic and euphallic individuals, respectively, with the number of individuals studied in parentheses. *P* is the *p* value of Fisher's exact test.

cated that strong genetic components were involved in the determination of aphally. The precise nature of these components cannot be specified though the determination is certainly complex since the two sexual morphs produced various family aphally ratios.

Reaction norms to temperature: The reaction norm of each lineage to temperature (Figure 4) clearly showed an increase of the aphally ratio with an increase of temperature. This effect seemed continuous with no threshold temperature since the two sexual morphs were produced at all temperatures. The relative values of the lineages for their family aphally ratio were similar for the three temperatures. The results of the analysis of deviance (Table 4) failed to show any significant interaction between temperature and lineage, temperature and lineage being the only significant effects. We therefore considered that all individuals within this population have a similar sensitivity to temperature but differ in their mean performance. The absence of a significant morph effect (Table 4) indicated that the two sexual morphs from a same lineage did not differ for their family aphally ratio. This was not surprising since the individuals were the products of three generations of selfing. The determination of the sexual morph within a family among the selfed G_4 offspring was probably not due to genetical differences.

Significant effects of individual, lineage and temperature were also observed for the survival rate. The mean survival rates (from eggs to phally determination) were 0.71 (SE = 0.31), 0.71 (SE = 0.29) and 0.60 (SE = 0.35) for 19°, 25° and 30°, respectively. This might have introduced a bias in the analysis of the aphally ratio provided that the two morphs suffered from differential mortality. In this case, the difference of aphally ratio between the temperatures and the lineages could be due to differential mortality. This hypothesis was tested by comparing the deviance of a factorial model involving temperature and lineage effects with the deviance of the same model after introducing offspring survival as a covariate (survival data arcsine transformed). The addition of this covariate had no significant effect (F [1;98] = 1.16, $P > 0.05$) and did not modify the significance of the other terms (interaction between temperature and lineage: F [18,98] = 1.61, $P > 0.05$; temperature: F [2,116] = 6.964, $P < 0.001$; lineage: F [9,116] = 29.96, $P < 0.001$). The variation in the aphally ratio cannot be explained by variation in the survival rate.

DISCUSSION

Our experiments clearly indicate that genetic components determine the sexual morph in *B. truncatus* with

TABLE 3

Level of genetic determination of aphally in four populations estimated using intraclass correlation and parent-offspring regression

	Intraclass correlation coefficient				Parent-offspring regression		
Population (aphally ratio)	No. of families	ANOVA 1	ANOVA 2	Probands	No. of lineages	Slope 1	Slope 2
Mari (0.78)	54 (614)	0.349 (0.054)	0.756 (0.116)	0.579	17	0.684 (0.209)	0.722 (0.153)
Namaga PM (0.69)	28 (712)	0.162 (0.045)	0.302 (0.084)	0.147	10	0.869 (0.232)	1.244 (0.499)
Namaga B (0.53)	77 (1039)	0.304 (0.042)	0.479 (0.066)	0.426	70	0.587 (0.116)	0.605 (0.120)
Dyoro (0.43)	16 (170)	0.332 (0.101)	0.561 (0.171)	0.592			

The intraclass correlation coefficients and the slope of the parent-offspring regression are estimated both from the dimorphic character (ANOVA 1 and Slope 1) and the underlying continuous variate of the threshold model (ANOVA 2 and Slope 2, probands methods). SEs of the correlation coefficients and slopes are given in parentheses. The number of families analyzed for the estimation of the intraclass correlation coefficient is given for each population with the number of offspring checked for the sexual morph in parentheses. The number of lineages used for the parent-offspring regression is also given for each population except for Dyoro for which the experiment did not cover two generations.

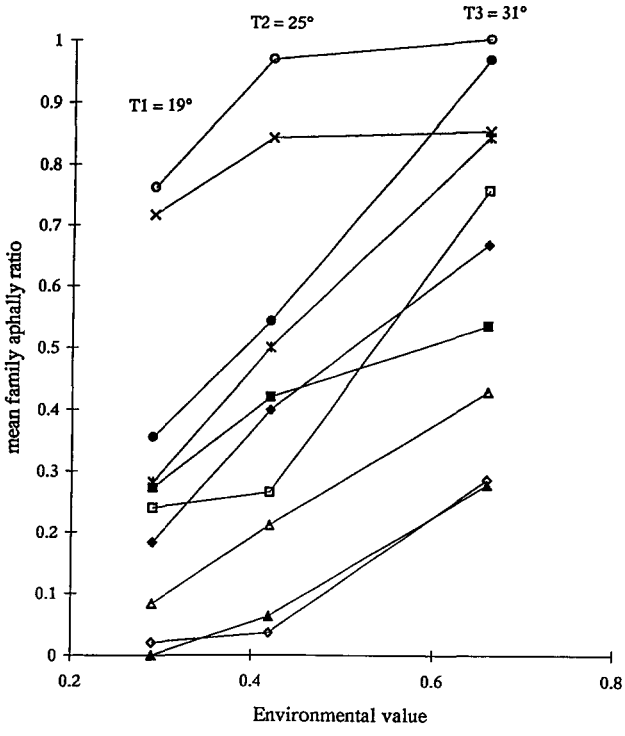

FIGURE 4.—Reaction norm to temperature. Each line is the reaction norm to three temperatures of one lineage estimated on the mean family aphally ratio. Ten lineages from Mari were tested. The environmental value is the mean aphally ratio over all lineages at a given temperature.

a high level of genetic determination in populations showing some variability for the determination of aphally. Various aphally ratios are produced in the progeny of individuals whatever their sexual morph, suggesting that many genetic factors are involved. We also observed a high sensitivity of the family aphally ratio to temperature; this ratio increasing with an increase in the rearing temperature. A factorial analysis of deviance showed that genetic and environmental factors are highly significant but do not interact, indicating that the lineages studied differ by their mean performance though not by their sensitivity to temperature. Recall that an individual reaction norm is characterized by its sensitivity to temperature (slope of the regression line of its family mean aphally ratio on temperature) and its mean performance (the mean of its family aphally ratio over the three temperatures).

LARAMBERGUE (1939) was the first to point out that aphally has a complex inheritance and that it is necessary to consider the heritability of the offspring aphally ratio rather than that of the parental sexual phenotype. This author was able to establish in some populations, but not in others, two lineages characterized by a very low and a very high aphally ratio in their offspring respectively. On the other hand, SCHRAG *et al.* (1992) failed to demonstrate any heritability of the sexual morph through a breeding and selection experiment. However, the individuals used in these two experiments were from a laboratory population originally based on only eight individuals. The genetic variability of this population was probably too limited to demonstre any heritability of aphally. Comparing the response to temperature of different populations of *B. truncatus*, SCHRAG *et al.* (1994b) showed a significant interaction term between population and temperature. However,

TABLE 4

Results of the analyses of deviance on aphally ratio and survival in the reaction norm experiment

Factor	Change in deviance	d.f.	F test	Deviance of the current model
		Aphally ratio		
Temperature (t)	268.90	2	72.63***	t.g + m + m.t + r + e
Lineage (g)	465.12	9	27.91***	t.g + m + m.t + r + e
Temp. line (t.g)	48.112	18	1.57	m + m.t + t + r + e
Morph (m)	0.897	4	0.13	m.t + r + e
Morph. temp (m.t.)	1.752	2	0.58	e
Parent (r)	68.66	29	1.56	e
Error (e)	97.177	64		
		Survival		
Temperature (t)	47.25	2	3.26*	t.g + m + m.t + r + e
Lineage (g)	432.29	9	6.64***	t.g + m + m.t + r + e
Temp. line (t.g)	67.43	18	0.48	m + m.t + r + e
Morph (m)	9.83	5	0.24	m.t + r + e
Morph. temp (m.t.)	0.28	2	0.03	e
Parent (r)	597.25	35	4.12***	e
Error (e)	322.776	78		

d.f. refers to the number of degrees of freedom, and the deviance of the current model refers to the deviance terms included in the denominator of the F test. ***$P < 0.001$; *$P < 0.05$.

in their experiment, the offspring from different individuals were tested at each temperature. Therefore, their results can reflect either the presence of individuals with different sensitivities or different mean performances between populations. Taking into account the high variability of the family aphally ratio and the absence of genetic-by-environment interaction observed in our experiment, we propose to interpret the determination of aphally in the context of the environmental threshold model developed by HAZEL *et al.* (1990).

The assumption of an underlying variate has frequently been invoked in the study of the heritability of binary characters having both environmental and genetic determination, such as the sex-ratio in reptiles (BULL *et al.* 1982; JANZEN 1992) and wing dimorphism in insects (ROFF 1986a,b; MOUSSEAU and ROFF 1989). This underlying variate may be a hormonal factor involved in the determination of sex (CREWS *et al.* 1994) and can also be invoked for wing dimorphism (HARRISON 1980). In the case of aphally, we do not have any indication of the physiological mechanisms involved in the determination of the sexual morphs. To explain our results on the genetical and environmental effects, the threshold model (see Introduction) can be modified to include an environmental cue (HAZEL *et al.* 1990; ROFF 1994). Transposing this model to aphally, the environmental cue is temperature. The genotype of an individual determines the temperature threshold value at which the dimorphic phenotype switches from one morph to the other (Figure 5, top). This temperature threshold value could be determined by the quantity of a hormonal factor. Threshold values are normally distributed in the population (Figure 5, middle). The higher family aphally ratio of aphallic individuals observed in some populations could be characterized by a lower mean of the distribution of the temperature threshold value (Figure 5, middle). If we consider that the genotype of an individual only determines the temperature threshold value (Figure 5, top), genetically similar individuals will have the same sexual phenotype at a given temperature. However, our results show that this is false. First, the reaction norm experiment shows that the two sexual morphs originating from a selfed lineage are genetically similar for the determination of aphally, since they did not differ in their family aphally ratio (no morph effect in the analyses of deviance). Second, we can consider that there is no genetic variability for the determination of aphally in populations with extreme aphally ratios. However, we always observed offspring with a sexual phenotype opposite to the most common one, when a large number of individuals was checked for aphally. Even under the same rearing temperature, individuals with the same genotype can differentiate into the two sexual morphs. This requires that other nonspecific environmental factors (which do not act as cues determining the sexual morph), such as developmental noises, are involved in

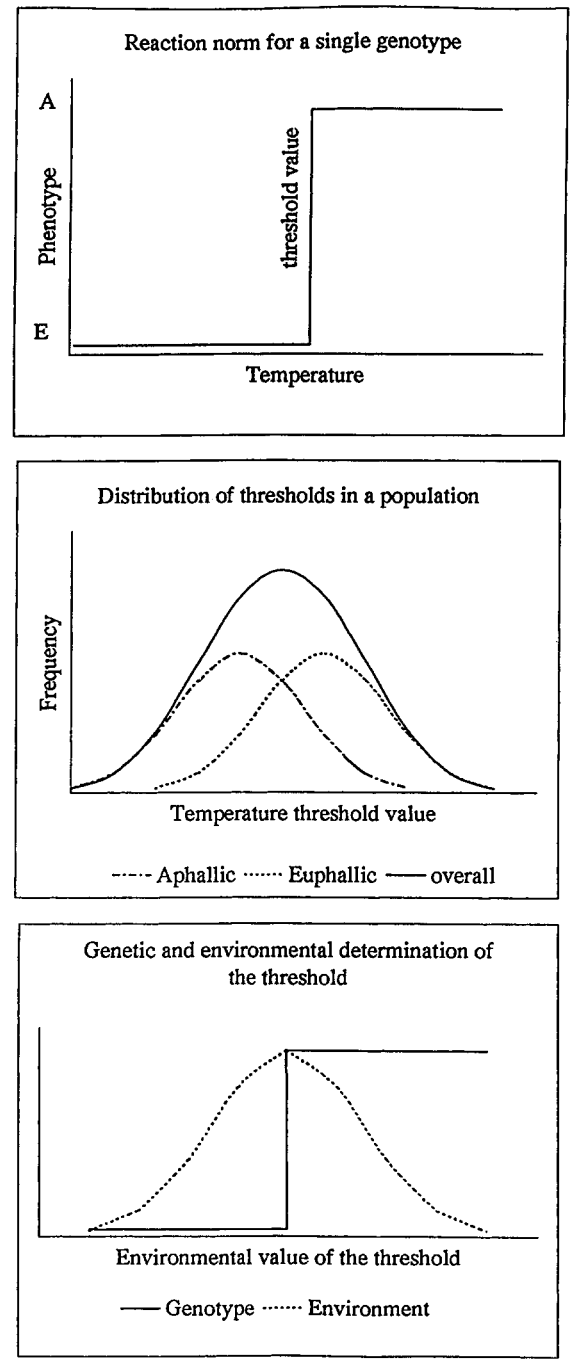

FIGURE 5.—The temperature threshold model for aphally (adapted from ROFF 1994). (Top) Each genotype is characterized by a temperature threshold value above which offspring are aphallic. A, aphallic; E, euphallic. (Middle) The threshold values are normally distributed in the population. The mean of the distribution is lower for aphallic than euphallic individuals at one temperature, *i.e.*, at a given temperature aphallic individuals will produce more aphallic offspring. (Bottom) The temperature threshold value can be determined both by the genotype and some environmental factors such as developmental noise (which do not act as an environmental cue such as temperature does). At a constant temperature, genetically similar offspring can have different sexual morphs according to this developmental noise.

the determination of the temperature threshold value (Figure 5, bottom). These environmental factors help explain that an inbred lineage can produce offspring of both morphs under laboratory conditions. Testing more specifically the occurrence and magnitude of this probabilitic process requires a comparison of the family aphally ratio under laboratory conditions of genetically similar individuals, which could be obtained after some generations of selfing. Controlled reciprocal crosses (see VAN DAMME 1983) between lineages characterized by different aphally ratios would help to determine the kind of inheritance (nuclear and/or cytoplasmic) of the genetic components involved in the determination of the temperature threshold value.

SCHRAG and READ (1992) argued that temperature sensitivity in the determination of aphally can be an adaptive character. In similarity with environmental sex determination (CHARNOV and BULL 1977), the sensitivity of phally determination to temperature can have evolved and be adaptive if (1) the factors determining the relative fitness of the two sexual morphs vary and are correlated with temperature and (2) the parents and the offspring have no possibility to choose the temperature the offspring will experience. Adaptive environmental sex determination has been supported for some species (CONOVER and HEINS 1987; NAYLOR *et al.* 1988). Condition (2) may be met in *B. truncatus,* and SCHRAG and READ (1992) proposed that changes in temperature may correlate with other changes in the environment, especially with population densities and parasitism that could affect differentially the fitness of the two morphs. To test this hypothesis, it is necessary to find genetical variation in sensitivity to temperature and to compare this variation to various population characteristics such as temperature variation. On the other hand, under the environmental threshold model, ROFF (1994) predicts a genetic correlation of one between the family mean ratios of the dimorphic traits across environments differing in only one factor, which is the case in our study. In other words, there must be no interaction between environmental and genetic factors. This prediction was experimentally observed in a study of wing dimorphisms in insects (ROFF 1994). The absence of a genotype-by-environment interaction observed in our reaction norm experiment agrees with this prediction. However, this result is based on only one population. Clearly, more investigations are required to test for the presence of genetic variation in sensitivity to temperature using individuals from various populations.

Populations of *B. truncatus* show very low allozyme variability. This has been explained by the population dynamics of this species characterized by frequent bottlenecks and high selfing rates (see NJIOKOU *et al.* 1993). It is therefore quite striking that we observed a high genetic variability for the determination of aphally within some populations. The question is then how this quantitative genetic variation can be maintained under the hypothesis of a polygenic determination of aphally and an environmental threshold model. Solutions for maintaining some quantitative genetic variation, such as a balance between mutation and selection, have been proposed for outbreeding populations (BARTON and TURELLI 1989) and highly inbred populations (CHARLESWORTH and CHARLESWORTH 1995). Moreover, in *B. truncatus,* the higher variability observed using microsatellites when compared to allozymes (F. VIARD and P. JARNE, unpublished results) suggests that some genetic variability for the determination of aphally may be maintained provided that the loci determining the temperature threshold value have a mutation rate higher than allozymes.

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