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Evolution, Vol. 46, No. 6 (Dec., 1992), 1698-1707.

Stable URL:
http://links.jstor.org/sici?sici=0014-3820%28199212%2946%3A6%3C1698%3ATDOMOA%3E2.0.CO%3B2-J

Evolution is currently published by Society for the Study of Evolution.

Evolution, 46(6), 1992, pp. 1698–1707

TEMPERATURE DETERMINATION OF MALE OUTCROSSING ABILITY IN A SIMULTANEOUS HERMAPHRODITE

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Abstract.—Phally, a genital dimorphism found in some species of self-compatible simultaneous hermaphrodites, presents an opportunity to examine factors maintaining outcrossing within an animal species in the presence of recombination. Both aphallics and euphallics can self-fertilize but only euphallics develop a functional penis and prostate allowing them to donate sperm. Previous studies of phally in the gastropod *Bulinus truncatus* (Mollusca: Pulmonata) suggest that phally may be under direct genetic control in some populations and strongly influenced by environmental factors in others. Experiments reported here identify temperature as a cue affecting phally determination in two populations of *B. truncatus*. In both populations, a higher proportion of euphallics was produced at low temperature (22 ± 1°C) than at high temperature (30 ± 1°C). Temperatures experienced by parents did not affect the proportion of euphallics they produced. Instead, phally was sensitive to temperature during the egg stage postoviposition and during the hatchling stage; the relative influence of temperature before and after hatching varied between populations. The total number of hatchlings reaching maturity at high and low temperature did not differ, but at low temperature, snails took longer to hatch and mature, and had lower survivorship. Just as studies of environmental sex determination have shed light on selective pressures influencing sex ratio evolution, we suggest that temperature-sensitive phally determination may shed light on the selective pressures maintaining outcrossing in *B. truncatus*.

Key words.—Aphally, *Bulinus truncatus*, evolution of sex, outcrossing, temperature sensitivity.

Received November 12, 1991. Accepted June 7, 1992.

The role of the environment is central in competing theories for the maintenance of sexual reproduction. Scenarios for a selective advantage of sex include uniform environments that vary temporally (e.g., Fisher, 1930; Williams, 1975), stable environments that vary spatially (e.g., Bell, 1982), and biotic environments that vary temporally (e.g., Hamilton, 1980). Other theories, particularly those concerning DNA repair (e.g., Bernstein et al., 1981), postulate no particular role for environmental factors beyond their influence on mutation rate. In this study we present evidence that environmental conditions during egg and hatchling stages directly influence male outcrossing ability in the gastropod, *Bulinus truncatus*.

Phally, a genital polymorphism found in several families within the Pulmonata (Mollusca: Gastropoda), presents an opportunity to examine factors maintaining outcrossing within an animal species in the presence of recombination. Some species of self-compatible simultaneous hermaphrodites have two sexual morphs, euphallic and aphallic. Euphallic individuals develop fully functional male and female tracts. In aphallics, the distal portions of the male tract never fully develop although functional sperm is still produced by the hermaphrodite gland (Geraerts and Joosse, 1984). Consequently, aphallics are not capable of sperm donation. Regardless of how often aphallics receive sperm [de Larambergue (1939) and Pokryszko (1987) report evidence that they do so], the outcrossing rate in such dimorphic populations must vary with the proportion of euphallics.

In natural populations of *Bulinus truncatus* (Audouin), the proportion of euphallics varies from zero to one (de Larambergue, 1939; Brown and Wright, 1972). Previous studies suggest that phally may have a strong heritable component in some (de Larambergue, 1939) but not all (Schrag et al., 1992) populations. In controlled laboratory populations of *B. truncatus*, Schrag et al. (1992) found more variability in the proportion of euphallics than predicted by chance alone suggesting an environmental component to phally determination. The present study demonstrates that temperature strongly influences phally determination in two populations of *B. truncatus*. Further experiments investigate the devel-

opmental period during which phally is temperature sensitive and measure life-history characters at high and low temperatures.

Environmental sex determination has been reported in both vertebrates and invertebrates (Naylor et al., 1988 and references therein). Labile sex determination is favored when fitness as a male or female varies predictably with the environment in which individuals develop and neither parent nor offspring has control over that environment (Charnov and Bull, 1977). We suggest that in the same way, environmental phally determination will be favored when fitness as a euphallic or aphallic is influenced in a predictable way by environmental conditions, thus providing a context for direct investigation of short term ecological factors maintaining outcrossing in *B. truncatus.*

Breeding Biology of B. truncatus

B. truncatus, an intermediate host for *Schistosoma haematobium,* is a tetraploid planorbid that inhabits a range of freshwater habitats from lakes to temporary pools in northern and western Africa and the Middle East (McCullough, 1962; Brown and Wright, 1972). We follow the nomenclature of Jelnes (1986) who gives synonyms of *B. truncatus.*

Our two laboratory strains derive from two dams 20 km apart in northern Nigeria. Both dams undergo marked seasonal changes in water level, vegetation density, and temperature (described in Betterton et al., 1988). Mean monthly air temperatures range from 21°C to 31°C (Olofin, 1987). The seasonal range of water temperature in an irrigation canal in northern Nigeria had a minimum of 13°C and a maximum of 32.5°C (Betterton, 1984). Diurnal fluctuation in water temperature increases when water levels are low (Betterton, 1984).

Snails begin to breed approximately four weeks after hatching. Both euphallic and aphallic *B. truncatus* can self-fertilize (de Larambergue, 1939). *B. truncatus* breeds continuously, although in the wild periods of aestivation can interrupt reproductive activity (Betterton et al., 1988). Eggs are laid in masses, typically of five or six eggs. Population density of *B. truncatus* reaches its minimum in the late rainy season (mean air temperature: 25.9°C) and its maximum in the middle of the dry season (mean air temperature: 23.7°C) (Betterton et al., 1988).

Materials and Methods

Experiments were carried out from October 1990 to August 1991 on snails from two populations. The Rimin Gado population of *B. truncatus,* derived from stock ETD No. 1483 maintained at the Natural History Museum, originated from a sample of eight snails collected at Rimin Gado Dam near Kano City in northern Nigeria in December 1987. The Kanyi population of *B. truncatus,* line ETD No. 1595, originated from a sample of 36 snails collected at Kanyi Dam, Kano, Nigeria in October 1990.

Rearing conditions (water, diet, and laboratory conditions) were described by Schrag et al. (1992). Experimental snails were chosen at random from the Rimin Gado and Kanyi populations before they reached 2 mm in length (prior to sexual maturity) and raised individually in 150 ml plastic pots. When isolated snails first produced eggs by self-fertilization, they were scored for phally and placed in one of four incubators according to randomly assigned temperature treatments. All four incubators were set at a 12L/12D photoperiod and constant temperature (±1°C). Incubator temperature treatments (high or low) were reversed between experiments to control for any variation between incubators. The water volume in parent pots was kept at 100 ml in all temperature treatments. The water in parent pots was changed once a week and the position of parents within incubators was randomized. Hatchlings (produced strictly by self-fertilization) were raised with their siblings in randomly positioned 450 ml pots (one hatchling pot per parent per temperature treatment). In all experiments, hatchling pots were cleaned when necessary with a minimum of one water change every two weeks. Hatchlings received a diet of freeze-dried lettuce and blue-green alga (*Oscillatoria* sp.). Adults were maintained on freeze-dried lettuce. All snails were provided with food ad libitum. Hatchlings reaching between 2 and 3 mm in length were scored nondestructively for phally as described by Schrag et al. (1992).

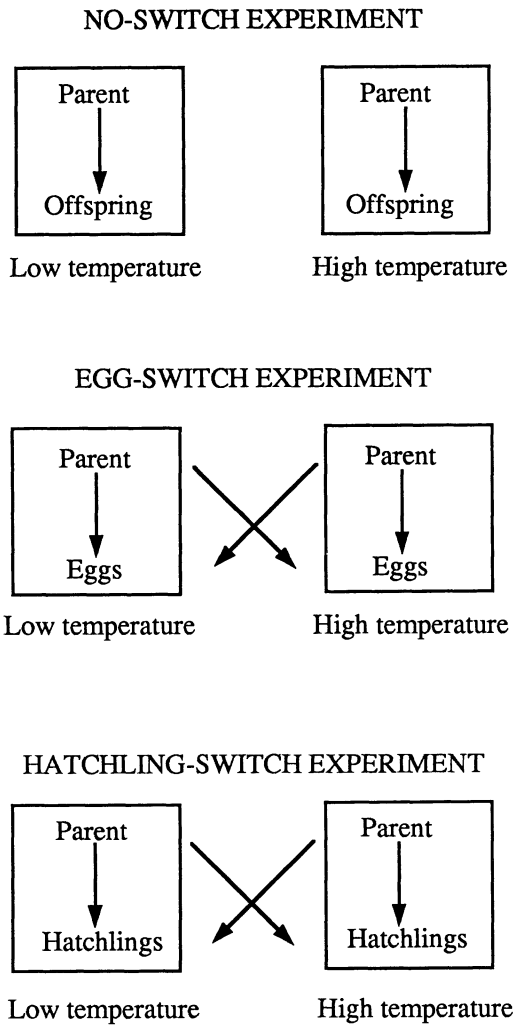


FIG. 1. Overall experimental design. For each experiment, one replicate of each treatment is shown. High (30 ± 1°C) and low (22 ± 1°C) temperature incubators were reversed between experiments to control for variation between incubators. In the egg-switch experiment, parents were from the Rimin Gado line. In the no-switch and hatchling-switch experiments, parents were from the Rimin Gado and Kanyi lines. In all experiments, offspring were raised and scored for phally.

The overall experimental design is diagrammed schematically in Figure 1.

No-Switch Experiments

These experiments were designed to determine whether temperature played a significant role in phally determination in two lines of *B. truncatus* by comparing the proportion of euphallics produced by snails maintained in low and high temperature incubators.

Two incubators were used in the no-switch experiments. Parents and their broods (defined as hatchlings resulting from a single parent) were maintained at either high temperature (30 ± 1°C) or low temperature (22 ± 1°C). New hatchlings were transferred once a week from parent pots to hatchling pots. Broods were raised and scored for phally. Experiments were carried out in two trials, the first on Rimin Gado snails and the second on Kanyi snails and can be divided into four groups according to line and temperature treatment (N = the number of broods/treatment): Rimin Gado snails raised at low temperature (N = 13); Rimin Gado snails raised at high temperature (N = 11); Kanyi snails raised at low temperature (N = 7); Kanyi snails raised at high temperature (N = 9).

One-Switch Experiments

The one-switch experiments were designed to determine the period of development during which temperature influenced phally determination. Three incubators (two high temperature and one low temperature) were used in these experiments.

Egg-Switch Experiment

The design of the egg-switch experiment allows comparisons of the proportion of euphallics resulting from transferred and control eggs in order to determine whether temperature prior to oviposition influenced phally determination. Parent snails consisted of 29 euphallics and 26 aphallics allowing analysis of the additional effect of parental phally on proportions of euphallics produced.

Approximately half of newly laid eggs produced by parent snails were transferred from high to low temperature treatment or vice versa (high temperature: 31 ± 1°C; low temperature: 22 ± 1°C). Remaining eggs were raised at the same temperature as their parents to serve as controls. Egg masses were transferred no more than 24 hours after they were laid. To facilitate egg transfer, pieces of polystyrene were introduced into parent pots as additional oviposition surfaces. Masses laid on the sides of parent pots were

gently dislodged and transferred using a paintbrush. Masses were allocated alternately to control or experimental treatments to avoid sampling bias. Parents with low fecundities (<10 eggs per week) were designated randomly either to control or experimental treatments to avoid small sample sizes in split broods. Control and experimental broods were raised and scored for phally. Egg-switch experiments were performed only on Rimin Gado snails and can be divided into four groups according to temperature treatment: broods raised from egg to maturity at low temperature (N = 19); broods in which newly laid eggs were switched from low temperature to high temperature (N = 21); broods raised from egg to maturity at high temperature (N = 21); broods in which newly laid eggs were switched from high to low temperature (N = 18).

Hatchling-Switch Experiment

The design of the hatchling-switch experiment allows within-line comparisons of the proportion of euphallics resulting in transferred and control hatchlings in order to determine whether temperatures before and after hatching had a significant effect on phally determination.

Approximately half of new hatchlings produced by parent snails were transferred from high to low temperature treatment or vice versa (high temperature: 30 ± 1°C; low temperature: 23 ± 1°C). Remaining hatchlings were raised at the same temperature as their parents to serve as controls. Hatchlings were transferred within two days of hatching. At any one time approximately half of new hatchlings were transferred to a new temperature treatment and half to the same temperature treatment. Parents with low fecundities (<10 eggs/week) were designated randomly either to control or experimental treatments early on to avoid small sample sizes in split broods. Control and experimental hatchlings were raised and scored for phally. Experiments were performed on both Rimin Gado and Kanyi snails simultaneously and can be divided into eight groups: Rimin Gado broods raised from egg to maturity at low temperature (N = 1); Rimin Gado broods in which newly hatched hatchlings were switched from low to high temperature (N = 10); Rimin Gado broods raised from egg to maturity at high temperature (N = 5); Rimin Gado broods in which newly hatched hatchlings were switched from high to low temperature (N = 15); Kanyi broods raised from egg to maturity at low temperature (N = 15); Kanyi broods in which newly hatched hatchlings were switched from low to high temperature (N = 18); Kanyi broods raised from egg to maturity at high temperature (N = 12); Kanyi broods in which newly laid eggs were switched from high to low temperature (N = 16).

Life-History Characters at Low and High Temperatures

The number of eggs and egg masses produced each week by parents in all four groups of the no-switch experiment was recorded from the time parents laid their first egg masses. In addition, the number of eggs that hatched and the number of hatchlings reaching 2.5 to 3 mm in length were recorded. Because phally is clearly distinguishable and egg production often begins when snails reach 3 mm in shell length (de Larambergue, 1939), snails reaching 3 mm were classified as mature.

The number of days for eggs to hatch was estimated in the hatchling switch experiment by monitoring three randomly chosen egg masses per parent. The number of days from egg to maturity was estimated by averaging across parents the number of days from first production of egg masses to first scoring of hatchlings. The mean density of hatchlings (per hatchling pot) was calculated as the average of weekly measures of cumulative hatchlings transferred minus hatchlings removed for scoring; this calculation will overestimate density because it does not take hatchling mortality into account.

RESULTS

Results are based on the phally of 1,116 hatchlings in the no-switch experiments (16 ± 0.9 hatchlings/parent; values reported as mean ± 1 SE), 812 hatchlings in the egg switch experiment (10 ± 0.6 hatchlings/half brood) and 1,429 hatchlings in the hatchling switch experiment (16 ± 1.2 hatchlings/half brood).

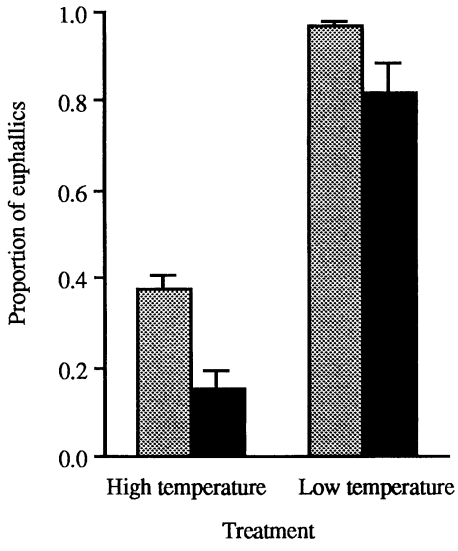


FIG. 2. Mean proportion of euphallics (± 1 SE) produced at high (30 ± 1°C) and low (22 ± 1°C) temperatures in the no-switch experiments; grey = Rimin Gado line, black = Kanyi line.

No-Switch Experiment

In the no-switch experiments, a higher proportion of euphallics was produced at low temperature in both lines (Fig. 2): Rimin Gado line, 0.38 ± 0.03 at 30°C, 0.97 ± 0.01 at 22°C (t_{21} = 15.8; P = 0.0001); Kanyi line, 0.15 ± 0.04 at 30°C, 0.81 ± 0.07 at 22°C (t_{14} = 6.7; P = 0.0001; unpaired t-tests calculated on arcsin transformed data, two-tail P values reported throughout). Between lines, the proportion of euphallics produced at high temperature and at low temperature is significantly different (30°C: t_{16} = 4.5, P = 0.0004; 22°C: t_{20} = 3.1, P = 0.005). This difference is probably due to a line × environment interaction. However, trials on Rimin Gado and Kanyi populations were carried out consecutively and incubator temperatures were reversed between experiments so we cannot test this formally; the between line differences could also be due to slight variation in conditions between experiments.

One-Switch Experiments

The between sibling correlation of a given variable, modified by sib relatedness (in this case greater than one half because sibs are the product of self-fertilization) estimates broad sense heritability of a trait (Falconer, 1981). Full sibling correlations often overestimate heritabilities due to shared environment and dominance components between siblings. In the present study, correlations of the proportion of euphallics in split broods are not significantly different from zero (egg switch experiment: high temperature versus high to low half broods: r = −0.55, N = 11, P = 0.08; low temperature versus low to high half broods: r = −0.05, N = 11, P = 0.88. Hatchling switch experiment: high versus high to low half broods: r = 0.24, N = 11, P = 0.48; low versus low to high half broods: r = 0.30, N = 14, P = 0.29). Thus, analysis of split broods in the egg switch and hatchling switch experiments provided no evidence of a heritable component to phally determination as expected from a previous study (Schrag et al., 1992). Consequently, in the following analyses split broods were treated as independent estimates of the proportion of euphallics in a given treatment.

Egg-Switch Experiment

The mean proportions of euphallics produced in the four treatments of the egg-switch experiment treatments are shown in Figure 3. The influence of temperature treatment before eggs were laid, temperature treatment after eggs were laid, and parental phally on the proportion of euphallics produced in broods was analyzed using a General Linear Model ANOVA on transformed data (Table 1). Parental phally does not influence the proportion of euphallics produced, supporting the results of Schrag et al. (1992). The only significant term in the model is temperature treatment after eggs were laid, which is highly significant (P < 0.0001). The mean proportion of euphallics in control broods and switched broods, compared according to the method of planned comparisons based on one-factor analysis of variance (Sokal and Rohlf, 1981), was significantly different (low versus low to high broods: $F_{1,75}$ = 137.0, P < 0.001; high temperature versus high to low broods: $F_{1,75}$ = 75.3, P < 0.001). When only temperature after laying is considered in the treatments shown in Figure 3, the mean proportions of euphallics produced in control and switched snails are similar to the high and low means in the no-switch experi-

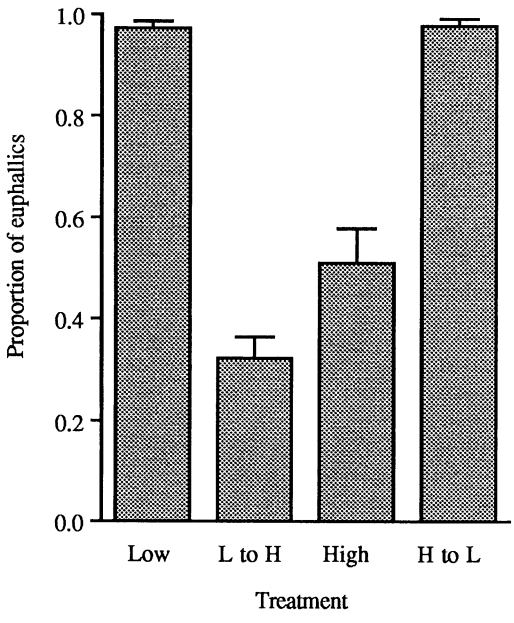


FIG. 3. Mean proportion of euphallics produced at high (31 ± 1°C) and low (22 ± 1°C) in the egg switch experiment. "H to L" and "L to H" represent high to low and low to high transferred broods.

ments. Incubator temperatures were reversed between the no-switch and egg-switch experiments, and the egg-switch experiment was performed three months after the no-switch experiment suggesting that there was no significant incubator effect on resulting proportions of euphallics.

Hatchling-Switch Experiment

The mean proportion of euphallics produced in the four hatchling-switch experiment treatments and the means broken down by line are shown in Figure 4. The influence of temperature before hatching, temperature after hatching, and line (Rimin Gado or Kanyi) on the proportion of euphallics produced was analyzed using a General Linear Model ANOVA on transformed data (Table 2A). Line is treated as a fixed effect preventing generalizations beyond the two lines studied. Because line and all but one of the higher interaction terms significantly influenced the proportion of euphallics produced, the influence of temperature on phally determination is analyzed separately for each line (Table 2B and C). In the Rimin Gado line, temperature before but not after hatching significantly influenced the proportion of euphallics produced. The interaction between temperatures before and after hatching is also significant; this results from the strong influence of temperature before hatching in low to high snails and temperature after hatching in high to low snails. In the Kanyi line temperature before hatching and temperature after hatching both significantly influenced the proportion of euphallics produced. The interaction term in this case is not significant, showing that temperatures before and after hatching contributed independently to the resulting proportion of euphallics.

TABLE 1. General linear model ANOVA on arcsin transformed proportion of euphallics in the egg-switch experiment. T_{bl} = temperature before eggs were laid; T_{al} = temperature after eggs were laid.

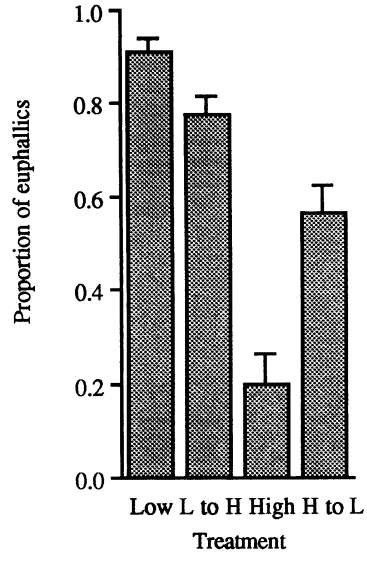
Source of variance	*df*	Adjusted sums of squares	*F*	*P*
T_{bl}	1	0.31	3.29	0.074
T_{al}	1	19.28	202.09	<0.0001
Phally	1	0.02	0.24	0.628
$T_{bl} \times T_{al}$	1	0.30	3.19	0.078
Error	72	6.86		

Life-History Characters at Low and High Temperatures

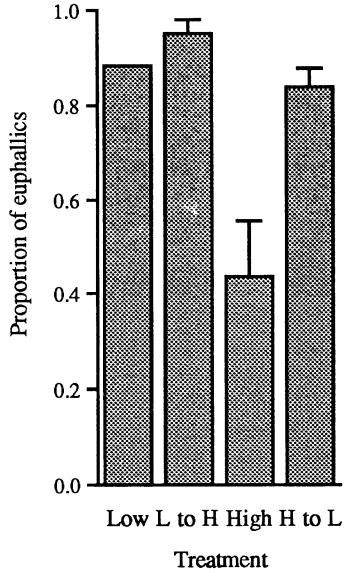
Mean values of life-history characters measured in the no-switch experiment are shown in Table 3. Means that differed significantly between lines are shown separately for each line.

Snails at low temperature lived longer, bred for longer, produced more eggs, and produced more egg masses than did snails at high temperature. However, a smaller proportion of eggs matured at low temperature offsetting this greater fecundity, so that the total number of hatchlings reaching maturity was the same in both temperature treatments. Eggs at low temperature took longer to hatch (more than twice as long as eggs at high temperature) and hatchlings at low temperature took longer to reach maturity.

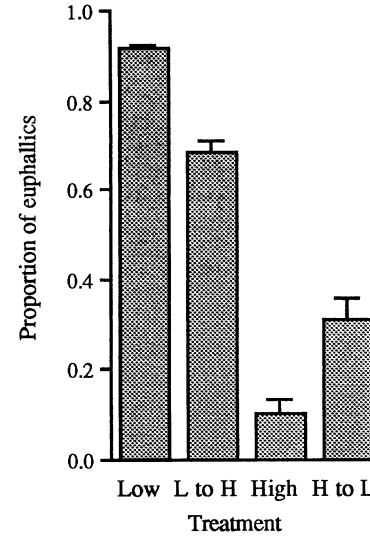
Between line differences may reflect between population differences in genotype although variation between experimental trials must also be taken into account. In both temperature treatments the Kanyi line lived and bred for longer than the Rimin Gado



(a) Overall



(b) Rimin Gado line



(c) Kanyi line

FIG. 4. Mean proportion of euphallics produced at high (30 ± 1°C) and low (23 ± 1°C) temperatures in the hatchling switch experiment. "L to H" and "H to L" represent low to high and high to low transferred broods.

line (weeks alive: $t_{35} = 4.3$; $P = 0.0001$; weeks breeding: $t_{35} = 3.0$; $P = 0.005$). The Rimin Gado snails bred continuously until they died while the Kanyi snails typically lived from one to three weeks post egg production before they died. Per week fecundity was higher in the Rimin Gado line (eggs/week: $t_{39} = 3.3$, $P = 0.002$; masses/week: $t_{36} = 3.2$, $P = 0.003$).

Discussion

A previous study of the heritability of phally provided the first evidence that phally in some populations of *B. truncatus* was under strong environmental control (Schrag et al., 1992). Experiments presented here identify temperature as a cue affecting phally determination and investigate the developmental time period during which phally determination was sensitive to temperature. At low temperature snails from two populations produced a higher proportion of euphallics than snails maintained at high temperature. Although our experiments show that phally was determined postoviposition, a parent's choice of oviposition site may affect its offspring's phally. Habitat selection by offspring after hatching may also play a role. In the Rimin Gado line, temperature after hatching did not significantly influence eggs incubated at low temperature while it did influence the magnitude of the proportion of euphallics resulting from eggs incubated at high temperature. In the Kanyi line temperature before and after hatching independently influenced the resulting proportion of euphallics. More lines would be required to analyze interpopulation variation in temperature-sensitive phally determination. Nonetheless, the results presented here demonstrate that temperature within a range snails naturally experience (Betterton et al., 1988) influenced phally determination in two populations of *B. truncatus,* one with a history in the laboratory and one newly collected from the field.

Environmental determination of phally has been demonstrated experimentally in *Deroceras laeve,* a temperate terrestrial slug with three genital morphs (Nicklas and Hoffman, 1981). By altering prehatching and posthatching conditions, Nicklas and Hoffman determined that phally in *D. laeve* was sensitive both to temperature and photoperiod before and after hatching. In contrast to the results presented here, euphally in *D. laeve* was apparently inhibited at cooler temperatures, though direct comparisons of the effect of temperature were made only for slugs maintained in total darkness. Reports of seasonal variation in the prevalence of euphallics in another land snail genus (*Zonitoides*: Watson, 1934) suggest that temperature-sensitive phally determination may not be limited to *B. truncatus* and *D. laeve.*

Aphally has evolved independently at least 14 times in the Pulmonata and is associated with a variety of ploidy levels, environments, and geographic areas (Schrag et al., in prep.). In some cases (e.g., within the genera *Vertigo* and *Vallonia*) it is found in several closely related species. The ability to donate sperm is unlikely to be adaptively neutral. Euphallics must pay a physiological cost of growing and maintaining a prostate and penis. Jarne et al. (1992) reported higher egg production in aphallics than in euphallics, which they interpreted as evidence

TABLE 2. General linear model ANOVA on arcsin transformed proportion of euphallics in the hatchling-switch experiment. A. Overall results. B. Rimin Gado line only. C. Kanyi line only. T_{bh} = Temperature before hatching; T_{ah} = Temperature after hatching.

Source of variance	*df*	Adjusted sums of squares	*F*	*P*
A.				
T_{bh}	1	3.39	56.25	<0.0001
T_{ah}	1	0.55	9.19	0.003
Line	1	1.10	18.32	<0.0001
$T_{bh} \times T_{ah}$	1	0.33	5.47	0.022
$T_{bh} \times$ line	1	0.36	6.04	0.016
$T_{ah} \times$ line	1	0.06	1.05	0.309
$T_{bh} \times T_{ah} \times$ Line	1	0.41	6.82	0.01
Error	83	5.00		
B.				
T_{bh}	1	0.46	6.29	0.019
T_{ah}	1	0.07	1.00	0.328
$T_{bh} \times T_{ah}$	1	0.44	6.06	0.021
Error	23	1.26		
C.				
T_{bh}	1	1.52	27.83	<0.0001
T_{ah}	1	9.15	167.76	<0.0001
$T_{bh} \times T_{ah}$	1	0.01	0.13	0.725
Error	57	3.11		

TABLE 3. Variation in life-history characters at high (30 ± 1°C) and low (22 ± 1°C) temperatures. Values are recorded as mean per snail ± 1 SE; t and P values based on two-tail unpaired t-tests; all proportions were arcsin transformed. Variables that differed significantly between lines are shown separately for each line. Snails reaching 3 mm in length were classified as mature.

Variable	High temperature	Low temperature	t	df	P
Total eggs produced	106.5 ± 17.1	185.3 ± 19.1	3.0	35	0.005**
Number of days for eggs to hatch	5.8 ± 0.1	12.2 ± 0.2	30.9	40	0.0001***
Total masses produced	21.4 ± 12.2	30.8 ± 11.9	2.4	35	0.02*
Mean density of hatchlings	76.9 ± 11.1	96.3 ± 10.2	1.3	31	0.22
Total hatchlings reaching maturity	23.9 ± 3.4	30.5 ± 4.1	1.2	38	0.23
Average survivorship from egg to maturity	0.33 ± 0.07	0.18 ± 0.0	−2.3	35	0.03*
Days from egg to maturity	28.8 ± 6.1	50.3 ± 13.7	6.0	38	0.0001***
Rimin Gado line:					
Weeks alive	3.1 ± 0.5	7.1 ± 0.8	4.1	19	0.0006***
Weeks of egg production	3.1 ± 0.5	6.8 ± 0.8	3.9	19	0.001**
Eggs produced per week	25.2 ± 3.3	24.6 ± 9.1	−0.1	20	0.90
Masses produced per week	5.4 ± 0.6	4.3 ± 1.4	−1.5	20	0.14
Kanyi Dam line:					
Weeks alive	6.0 ± 1.2	11.9 ± 1.9	4.4	17	0.0003***
Weeks of egg production	5.1 ± 1.0	9.0 ± 0.4	4.1	19	0.002**
Eggs produced per week	19.4 ± 2.4	13.6 ± 1.7	−2.0	17	0.06
Masses produced per week	4.3 ± 0.4	2.3 ± 0.6	−5.1	14	0.0002***

* Significant at the 0.05 level; ** significant at the 0.01 level; *** significant at the 0.001 level.

of the cost of euphally. Furthermore, snails whose eggs are fertilized by allosperm as opposed to autosperm pay the additional cost of sex (Maynard Smith, 1978). Consequently, euphallics must be maintained within populations of aphallics only when outcrossing is beneficial.

Environmental sex determination has shed light on the adaptive significance of sex allocation in a number of species (i.e., Conover and Heins, 1987; Tingley and Anderson, 1987; Naylor et al., 1988; Janzen and Paukstis, 1991). Similarly, temperature-sensitive phally determination raises the following question: if the frequency of aphally is under selection, why might phally determination by temperature-dependent? One possibility is that outcrossing is favored at low temperatures where generation times are longer, and offspring are thus more likely to experience conditions unpredictably different from those experienced by their parents (Williams, 1975). In our study, eggs at low temperature took twice as long to hatch and 1.7 times as long to reach maturity as eggs at high temperature. In the absence of comprehensive field data, it is difficult to assess whether such an increase is sufficient to generate a selective advantage to outcrossing. However, this idea would not explain Nicklas and Hoffman's (1981) observation that euphally in *D. laeve* increased at high temperature [which corresponds with shorter generation time (Riddle, 1983)].

Changes in temperature may correlate with other changes in the environment and it may be that one of these factors, rather than temperature per se, exerts a selective pressure favoring outcrossing. In species with intermittent sexuality, environmental cues, in particular limited resources and high population density, are often associated with prevalence of the sexual form (Bell, 1982). In our experiments, mean density in hatchling pots in both treatments was not significantly different and food was always provided in excess, suggesting that if density and nutritional status influence the selective advantage of outcrossing, temperature is still a proximate cue. In the field, populations of *B. truncatus* reach their maximum density during the coldest months (Betterton et al., 1988) so temperature could serve as an adaptive cue for population density. Low temperature might also be associated with an increased likelihood of parasitism, another scenario for a selective advantage of outcrossing (Hamilton, 1980; Lively, 1987). *B. truncatus* is known to be the host for at least 12 different species of cercariae (Ndifon and Umar-Yahaya, 1988–1990). Appropriate field studies of temperature vari-

ation, and ecological and behavioral correlates of high and low temperatures, should directly address the short term ecological factors maintaining outcrossing in this species.

Acknowledgments

We are grateful to J. Howe for technical assistance, D. Rollinson and G. T. Ndifon for supplying both snail populations, the E. P. A. Cephalosporin Fund for covering the cost of two incubators, and J. Bergelson and J. Newman, J. Bull, D. Haig, P. Harvey, A. Keymer, and D. Rollinson for helpful discussion and/or comments on the manuscript. S.S. was supported by a Marshall Scholarship, A.R. by a Junior Research Fellowship from Christ Church and a Lloyd's of London Tercentenary Fellowship.

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