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HUMIDITY AND THE EFFECT OF SHELL COLOUR ON ACTIVITY OF *CEPAEA NEMORALIS* (LINNAEUS, 1758)

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Abstract: Activity of brown and yellow unbanded shelled *Cepaea nemoralis* (L.) was observed in 23 outdoor cage experiments carried out at various weather conditions in Słupsk, northern Poland. The experiments were carried out at noon local time and lasted one hour; the activity of each snail was scored at 5 minute intervals. In the range of temperatures examined in this study, air humidity was the significant factor associated with differences in the activity of the morphs. At air humidity of approximately 90%, all snails remained active throughout the experiment, irrespective of the colour of the shell. At air humidity of over 70% brown snails remained active significantly longer than yellow ones. When humidity was below 70%, yellow snails remained active significantly longer than brown ones, and the onset of inactivity was earlier with decreasing air humidity. No consistent effect of the level of solar radiation, air temperature, or air humidity/temperature ratio was observed. Even though the differences in the time of remaining active were not large, they indicate that yellow and brown *C. nemoralis* are adapted to different climatic conditions.

Key words: *Cepaea nemoralis*, animal coloration, polymorphism, snail behaviour

INTRODUCTION

Cepaea nemoralis is a polymorphic land snail species with yellow, pink or brown shells bearing up to 5 bands. Variation among populations can be very large, but light-coloured snails are usually associated with hot dry regions of the species distribution, and within a region – with open sunny habitats, whereas dark-coloured snails are associated with cooler regions and shaded habitats; this pattern is often explained in terms of climatic selection (Jones et al. 1977, Ożgo 2005).

In many poikilothermic animals, absorption of solar energy and internal temperatures are affected by pigmentation. In *C. nemoralis*, differences in pigmentation of various morphs lead to significant differences in internal temperatures, both in live snails and in mercury-filled shells. The greatest differences were found between the most contrasting morphs: when exposed to direct solar radiation, brown unbanded snails heated up more and reached internal temperatures approximately 1°C higher than yellow unbanded ones (Heath 1975). Similarly, Tilling (1983) found that internal temperatures in brown unbanded morphs were significantly higher than in yellow midbanded morphs throughout most of the daytime. A rise in temperature in dark-shelled snails, as compared to light ones, can lead to higher metabolic rates, increased activity, growth rate and fecundity. On the other hand, when exposed to intensive solar radiation dark snails are more liable to death through overheating and dehydration (Jones 1973, Heath 1975). In principle, such differences can be of selective importance. However, any difference in temperature due to colour can possibly be negated by differences in behaviour between morphs (Heath 1975).

Differences in the behaviour of various morphs are known to occur. Wolda (1965) has shown that different morphs of *C. nemoralis* differ in their climbing tendency. Jones (1982) has found differences in the duration of daytime activity between yellow unbanded and yellow five-banded phenotypes when exposed to sunlight. Tilling (1983) has demonstrated behavioural differences between artificial “morphs” (snails of the same natural morph painted black or white), the direction of which suggest that thermal relations may be responsible; concurrent observation of brown unbanded and yellow mid-banded snails revealed similarities between natural morphs and their artificial mimics, indicating a mechanistic response of the snails. Differences in the time of remaining active between banded and unbanded *C. nemoralis* were observed by CHANG (1991).

Becoming inactive is thought to be one of behavioural adaptations for avoiding thermal stress and dehydration in terrestrial snails (CHANG 1991); water loss from inactive snails is at least three orders of magnitude less than rates observed in active ones (MACHIN 1975). Therefore, differences in the time of remaining active can indicate differences in the resistance to environmental stress. In the present study, two questions have been addressed: 1. Are there differences in the time of remaining active between different coloured snails? 2. What conditions limit the activity of either morph? In order to answer these questions we have modified the experiments designed by CHANG (1991) and carried them out in a broad range of weather conditions.

MATERIAL AND METHODS

Activity of brown unbanded and yellow unbanded *C. nemoralis* was observed in 23 experiments carried out in summer months 2003 and 2004 in Słupsk (N 54°28’, E 17°01’), northern Poland. In 2003, 55 brown and 103 yellow snails were used, in 2004 there were 105 and 110 snails, respectively. The number of snails of each morph observed in one experiment ranged from 7 to 30. The snails originated from two colonies, as we did not succeed in finding one with sufficient numbers of both forms. Brown snails were collected in Słupsk, and yellow ones in Poznań (220 km south of Słupsk) – the closest by place where yellow unbanded *C. nemoralis* are known to be frequent (WAGNER 1990). The snails inhabited similar semi-open habitats. There was no difference in shell size between the morphs. The observations were carried out either on clear or on overcast (including rainy) days, but not on days with partly overcast sky or with unstable weather. All the experiments were carried out at noon, so that the effect of a diurnal rhythm might be neglected. Also, as the main problem of terrestrial snails is avoiding overheating and dehydration, any differences in activity were expected to show particularly clearly at this time of the day.

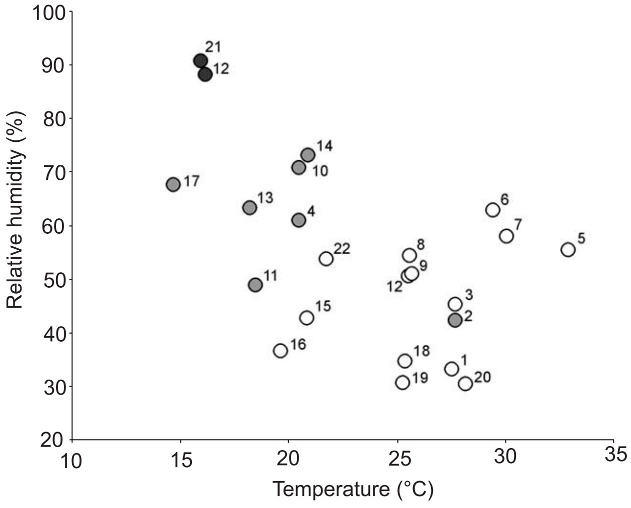

Fig. 1. Average weather conditions during the experiments. Black circles, rain; grey circles, overcast; white circles, sunny. Numbers indicate numbers of experiments

The snails were kept in an outdoor cage and provided with a supply of food. Prior to each experiment, equal numbers of yellow and brown snails were chosen at random and immersed in water until all individuals became active; they were then moved to one or two (depending on the number of snails) experimental cages made of 1.8 cm^2 green plastic mesh (height 50 cm, diameter 32 cm) placed on a short grass lawn in an open place. Dried tree sticks were put into the cages to provide climbing objects. Observations started half an hour before noon local time, and lasted one hour. At 5 minute intervals, each snail was scored as active, partly active or inactive. A snail was regarded active when it had at least one tentacle protruding; partly active – when its foot was slightly everted with the head and tentacles not protruding; inactive – when its body was completely withdrawn into the shell. In the present analysis partly active and inactive snails were considered together as the inactive category. Air humidity and ambient temperature readings were taken in shade 20 cm above the ground at the same 5 minute intervals, and average conditions for each experiment were calculated. The average temperature ranged from 15°C to 33°C, and average air humidity from 30% to 91% (Fig. 1). The level of solar radiation was approximated by the “clear sky” and “overcast sky” categories.

For each experiment, the differences in activity have been tested for significance by Wilcoxon’s paired sample test. When a number of tests are performed in a study, it is often demanded that Bonferroni correction be used in order to decrease type I errors (ŁOMNICKI 1999). However, this correction largely increases the likelihood of type II errors, meaning that important differences might be deemed non-significant. As the Bonferroni method is regarded to create more problems than it solves (PERENGER 1998) it has not been applied in this study.

RESULTS

Statistically significant differences in activity between yellow and brown unbanded *C. nemoralis* have been found in 11 out of 23 experiments. In 9 cases brown snails became inactive more quickly than yellow ones, and in 2 cases brown remained active longer than yellow. In 12 cases the differences were not significant. The results of individual experiments are given in Table 1.

In order to identify the factors causing differences in the activity of the snails, several possibilities were considered: intensity of solar radiation, air temperature, humidity, and simultaneous action of temperature and humidity.

Fourteen experiments were carried out on sunny days: in 7 cases yellow snails remained active significantly longer than brown ones, and in another 7 the differences were not significant. On overcast days 7 experiments were carried out: in 2 cases brown snails remained active significantly longer than yellow ones, in 2 cases yellow snails remained active significantly longer, and in 3 cases the differences were not significant. On rainy days (2 experiments) nearly all snails remained active throughout the experiments and there were no differences between the morphs.

Experiments 4, 5, 6, 7, 13 and 17 were carried out at a similar air humidity level (55.5% – 67.5%) but over a wide temperature range (14.7 – 32.8°C). In spite of the differences in temperature, snail activity in those experiments was strikingly similar. The time when all snails remained active lasted 30 to 45 minutes, and afterwards brown snails were becoming inactive more quickly than yellow ones (but the difference was statistically significant only in experiments 4

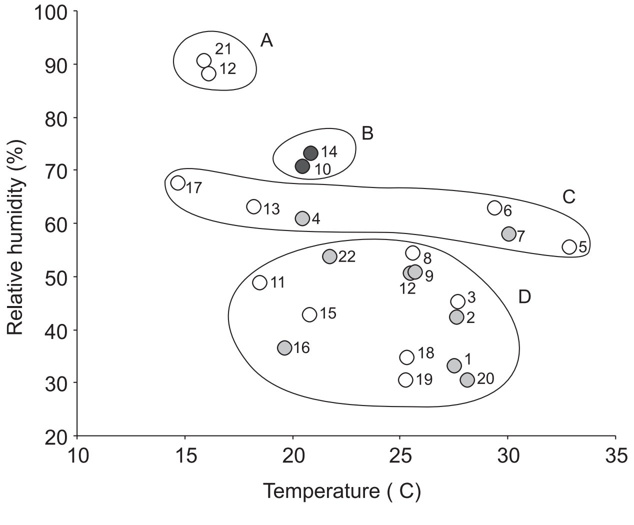

Fig. 2. Groups of activity patterns in the experiments. Dark circles, brown snails remaining active significantly longer than yellow ones; grey circles, yellow snails remaining active significantly longer than brown ones; white circles, differences not significant

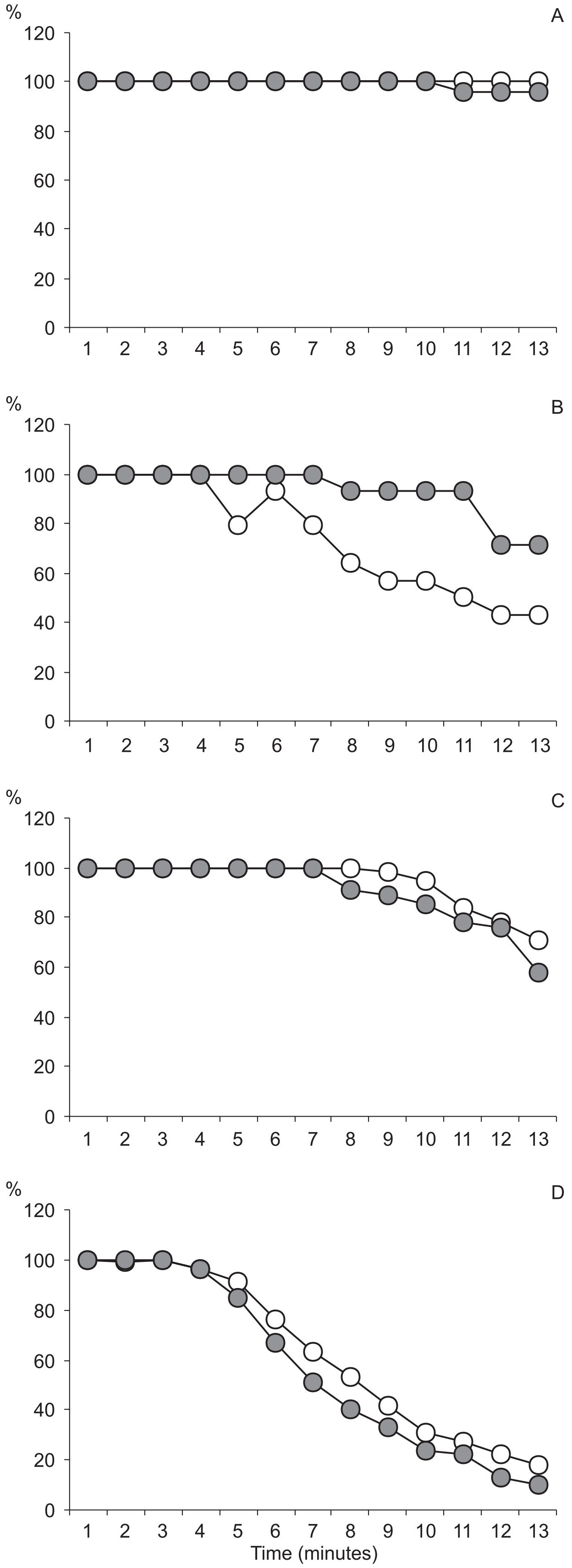

Fig. 3. Percentage of snails remaining active throughout the experiments. Data grouped as in Figure 2. Dark circles, brown snails; white circles, yellow snails

Table 1. Results of individual experiments. No – number of the experiment, S – sunny, O – overcast, R – rainy, Y – yellow unbanded, B – brown unbanded, value of p in Wilcoxon's paired sample test

No/date	S/O/R	Temp. °C	%RH	Morph	Minutes													p
					0	5	10	15	20	25	30	35	40	45	50	55	60	
1	S	27.5	33.1	Y	7	7	7	7	7	6	6	6	5	3	3	2	1	.008
18.06.03				B	7	7	7	6	7	5	5	5	4	2	2	1	0	
2	O	27.6	42.3	Y	7	7	7	7	7	7	6	5	4	3	2	0	0	.012
24.06.03				B	7	7	7	6	6	6	2	2	1	1	1	0	0	
3	S	27.7	45,2	Y	7	7	7	6	6	6	5	4	4	3	3	2	2	.398
20.06.03				B	7	7	7	7	5	3	2	4	3	3	3	3	3	
4	O	20.4	60.9	Y	7	7	7	7	7	7	7	7	7	6	5	5	5	.043
25.06.03				B	7	7	7	7	7	7	7	6	6	5	5	4	4	
5	S	32.8	55.5	Y	7	7	7	7	7	7	7	7	7	7	5	5	5	.686
01.08.03				B	7	7	7	7	7	7	7	7	6	6	6	6	6	
6	S	29.4	62.8	Y	7	7	7	7	7	7	7	7	6	7	7	6	5	.068
02.08.03				B	7	7	7	7	7	7	7	6	6	6	6	6	4	
7	S	30.0	58.0	Y	7	7	7	7	7	7	7	7	7	6	5	4	4	.028
03.08.03				B	7	7	7	7	7	7	7	4	5	4	3	3	3	
8	S	25.6	54.4	Y	7	7	7	7	7	6	6	6	5	3	3	1	0	1.000
04.08.03				B	7	7	7	7	7	6	6	5	4	2	3	2	2	
9	S	25.7	50.9	Y	7	7	7	7	7	6	6	6	5	3	3	3	3	.015
05.08.03				B	7	7	7	7	6	7	5	4	3	0	1	1	0	
10	O	20.4	70.8	Y	7	7	7	7	4	6	4	3	3	4	4	1	2	.008
24.08.03				B	7	7	7	7	7	7	7	6	6	6	6	4	4	
11	O	18.5	48.9	Y	7	6	7	7	7	7	6	5	4	4	3	2	1	.128
27.08.03				B	7	7	7	7	7	6	6	4	5	4	5	5	4	
12	R	16.3	88.1	Y	7	7	7	7	7	7	7	7	7	7	7	7	7	.109
30.08.03				B	7	7	7	7	7	7	7	7	7	7	6	6	6	
13	O	18.2	63.2	Y	7	7	7	7	7	7	7	7	7	6	6	4	3	.—
01.09.03				B	7	7	7	7	7	7	7	7	6	6	5	4	3	
14	O	20.9	73.1	Y	7	7	7	7	7	7	7	6	5	4	3	5	4	.028
11.09.03				B	7	7	7	7	7	7	7	7	7	7	7	6	6	
15	S	20.8	42.7	Y	20	20	20	20	20	18	13	11	7	6	5	4	3	.123
12.05.04				B	20	20	20	20	19	16	11	10	9	6	6	1	0	
16	S	19.6	36.5	Y	30	30	30	30	30	30	25	25	19	14	13	13	10	.008
14.05.04				B	30	30	30	30	29	27	20	19	15	13	10	6	3	
17	O	14.7	67.5	Y	20	20	20	20	20	20	20	20	20	20	18	19	17	.—
19.05.04				B	20	20	20	20	20	20	20	20	20	20	18	19	12	
18	S	25.3	34.7	Y	20	20	20	18	15	8	3	4	4	4	3	2	3	.646
01.06.04				B	20	20	20	20	16	13	8	6	3	2	0	0	2	
19	S	25.2	30.6	Y	20	20	20	20	18	14	12	5	2	2	1	2	3	.624
02.06.03				B	20	20	20	20	18	15	11	6	5	3	3	0	1	
20	S	28.1	30.5	Y	20	20	20	18	15	12	11	6	8	5	5	4	3	.013
04.06.04				B	20	20	20	19	14	8	7	6	4	3	2	0	0	
21	R	15.9	90.7	Y	20	20	20	20	20	20	20	20	20	20	20	20	20	.—
15.06.04				B	20	20	20	20	20	20	20	20	20	20	20	20	20	
22	S	21.7	53.8	Y	20	20	20	20	20	17	15	11	8	5	4	5	5	.012
22.06.04				B	20	20	20	19	16	11	9	5	3	3	4	4	5	
23	S	25.5	50.6	Y	20	20	20	18	15	8	7	8	6	5	3	3	1	.012
23.06.04				B	20	20	20	17	14	6	5	1	4	5	3	1	0	

Numbers of active snails in particular 5-minute intervals of experiments are given.

and 6); after one hour approximately 50% of the snails were still active.

Aggregating the results according to the air humidity gradient revealed four groups of distinct activity patterns (Figs. 2 and 3):

A. At air humidity of about 90% all snails remained active throughout the experiment.
B. At air humidity exceeding 70%, brown snails remained active longer than yellow ones.
C. At air humidity between 55 and 70% reaction time for both morphs was quite long, about 30 min, and after that time brown snails became inactive more quickly than yellow ones. After one hour approximately 50% of the snails were still active.
D. When air humidity fell below 55%, reaction time was only about 15 minutes, and after that the snails became inactive very quickly, with yellow remaining active longer than brown ones. At the end of those experiments only few snails were still active.

Arranging the results according to the humidity/temperature ratio does not reveal any consistent pattern in the activity of the snails.

DISCUSSION

The results of the present study show that the level of activity of brown and yellow *C. nemoralis* can differ significantly in certain weather conditions. The idea that the level of solar radiation is the differentiating factor is very suggestive, and seems to follow from previous studies (JONES 1973, HEATH 1975, TILLING 1983). However, this view is not really supported by our results. Although on clear-sky days yellow snails tended to remain active longer than brown ones, brown remained active longer than yellow in only two experiments out of 7 carried out on overcast days. In an experiment carried out on an overcast but a hot and dry day (experiment 2) brown snails became inactive much more quickly than yellow ones ($p=0.012$), and after one hour there were no snails of either morph remaining active. This pattern resembles other experiments carried out on hot and dry days, and not the pattern observed in other overcast–sky experiments, carried out in cooler and more humid conditions.

Temperature is another very likely factor, often invoked as causing differences in the distribution of morph frequencies in natural populations of the species (JONES et al. 1977). However, present results do not support this view either. There were six experiments carried out with a temperature range of nearly 20°C, and the pattern of snail activity in all those experiments was strikingly similar. In the experiment carried out on the hottest day (5) the overall difference in the activity of the snails was not significant, and after one hour there were still over 70% of snails of each morph remaining active. This pattern much more resembles the pattern observed on cooler days of comparable humidity than one observed on equally hot, but drier days.

The most consistent view is obtained when the results are arranged according to the air humidity gradient. At very high humidity values all snails remained active, irrespective of the colour of the shell. At air humidity of over 70% it was the brown snails that remained active significantly longer than yellow. However, there were only two experiments carried out in those conditions, and their results should be treated with some caution. At the humidity below 70% yellow remained active longer than brown, and the time of becoming inactive was shortening with decreasing air humidity. No effect of combined temperature and humidity gradient was observed.

Behaviour in sunlight is the prime mode of thermoregulation in invertebrates; in terrestrial snails it may involve seeking cooler or sheltered places, and possibly differentiate microhabitat choice of different morphs of *C. nemoralis* (JONES 1982). However, our results do not support the view that becoming inactive is in itself a way of avoiding thermal stress, as has been suggested by CHANG (1991). In the range of temperatures examined in this study, air humidity is the most likely factor causing differences in the activity of the morphs. This is broadly in agreement with the principle that it is the environmental water that plays the dominant role in regulating activity in terrestrial pulmonates (MACHIN 1975). It is not to say that other climatic components do not exert influence; snails almost certainly react to a combination of factors. Apart from the variables included in this study, it would be worthwhile to actually measure the level of solar radiation, and to examine the effect of wind and drying power. But it is also quite possible that snails respond to very subtle shifts in environmental factors, whose magnitude we are not always able to detect. The picture is further complicated by behavioural differences among individual snails.

Nevertheless, the results of this study show that weather conditions can affect the strength and direction of differences in activity between different morphs of *C. nemoralis*. Surely, the impact of humidity cannot be ignored in looking at differential activity. This finding might explain some aspects of the behaviour of those snails. For example, observations of *C. nemoralis* climbing trees revealed marked and often significant differences in the height reached by different morphs, but their direction differed among observations, giving puzzling results (WOLDA 1965, R.A.D. CAMERON, pers. comm.). Probably, repeatability of this behaviour might be observed under similar weather conditions; the absence of humidity records from the earlier works might be responsible for the inability to explain some contradictory results (e.g. WOLDA 1965).

Differences in the time of remaining active observed between different morphs suggest that they may be adapted to different climatic conditions. This might explain some distributional patterns observed both at a macro- and a micro-scale. For example, brown *C. nemoralis* are known to occur in high frequencies only in Britain (e.g. SHEPPARD 1952, CAIN & SHEPPARD 1950, CAIN 1968, GREENWOOD 1974), parts of Germany (SCHILDER & SCHILDER 1957) and northern Poland (unpublished data). In other parts of the species distribution they are rare or absent altogether. A possible explanation of this striking distribution pattern is that this morph is especially demanding as to the level of air humidity.

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