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The response of the terrestrial slug *Deroceas laeve* to the mucus and air-borne odours of con- and heterospecifics (Pulmonata: Agriolimacidae)

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Gastropods produce mucus for a number of reasons.¹ One of these is attracting potential mates through (volatile) metabolites that are released in the mucus.^{2,3} Hence, mucus may to a greater or lesser extent be responsible for influencing the behaviour of conspecific and even related heterospecific individuals. Moreover, interference competition through mucus of conspecifics and related heterospecifics may reduce growth rates and reproduction in individuals, thus affecting their fitness.⁴⁻⁶

We tested the response of the terrestrial land slug *Deroceas laeve* on the mucus of unstressed and stressed con- and heterospecifics, and on air-borne odours of conspecifics. *D. laeve* (Müller) individuals were obtained from a laboratory culture. *D. reticulatum* (Müller) and *D. panormitanum* (Lessona & Pollonera) were used for trials with heterospecifics. All individuals were kept isolated in plastic containers with a piece of sponge and damp paper towel. Dried cat food and poplar leaves were given as food. Animals were kept in a climate room at a constant temperature of 18°C and a 16 h light/8 h dark rhythm.

Only equally sized adult individuals were used. Individuals that were used to obtain stressed mucus were not used in the trials. Individuals were only used once in each experiment. All experiments were carried out in complete darkness. After each trial, all experimental equipment was cleaned with water and detergent to remove all mucus.

In a first experiment we tested the response of *D. laeve* on mucus of unstressed conspecifics using two different set-ups. In the first set-up, we used 12 × 17-cm plastic boxes that were surrounded with copper foil to prevent slugs from escaping. Half the surface of a box was lined with untreated damp paper towel and poplar leaves. The other half of a box was lined with damp paper towel and poplar leaves containing mucus from unstressed conspecifics. Unstressed mucus was obtained by letting three slugs move for 30 min in complete darkness in a 12 × 8.5-cm plastic box surrounded with copper foil. The bottom of the box was covered with damp paper towel and poplar leaves. The paper and leaves with the mucus were transferred to the larger experimental box. At the beginning of each trial one experimental animal was placed at the contact line of the two surfaces. The behavioural response of 30 slugs was filmed for one hour with a Sony DLR-TRV 130 E digital handycam attached to a VHS videorecorder in a climate room at 18°C. The 'super nightshot' option of the handycam allowed filming in complete darkness. Each time, two experimental boxes were filmed at the same time with a different orientation of the treated surface to avoid any bias from movements towards a certain direction. After 1 h we recorded the position of each slug, calculated the time that was spent on each surface and calculated the time that the individual was active on each surface. Activity was expressed as: (total time an individual was crawling in area/total time spent in area) × 100% and arcsin square root transformed to meet the parametric assumptions of normality.⁷ Individuals that showed no activity within 10 min of the start of the experiment were excluded from the analysis. The preference for one of the two areas was tested with a χ^2 test. Differences in the time spent on the two areas and differences in

activity on the two areas were tested with a paired Student's *t*-test. There was a tendency for individuals at the end of an hour to be on areas with mucus of conspecifics rather than on areas without mucus although the difference was not significant ($\chi^2 = 3.33$; *df* = 1; *P* = 0.06). Yet, the time spent on areas with mucus of conspecifics (41.70 ± 16.97 min) was significantly higher than the time spent on areas without mucus (17.28 ± 15.43 min; paired *t*-test: *t* = 4.45; *df* = 29; *P* < 0.001; Fig. 2A). The times spent active on both areas (mucus: 81.15 ± 29.93%; no mucus: 90.14 ± 20.25%) were the same (paired *t*-test: *t* = -0.96; *df* = 16; *P* = 0.35; Fig. 3A).

In a second set-up, glass horizontal Y-maze tubes were used (Fig. 1). Each tube consisted of an 80-mm stem and two 80-mm arms. Stem and arms had an internal diameter of 15 mm. The bottom of each tube was lined with damp paper towel and poplar leaves. Mucus trails were obtained by letting an individual crawl on a piece of damp paper towel with poplar leaves between two copper plates 15 mm apart. Untreated strips and strips containing conspecific mucus were alternately applied to the left and right arm of the Y-maze to avoid any bias of movements towards one of both arms. An experimental animal was placed in the stem of the Y-maze and we recorded which of the two arms was chosen. Individuals that did not enter one of the arms within 30 min were excluded from the analysis. We observed a strong preference for the arm containing mucus of a conspecific ($\chi^2 = 9.32$; *df* = 1; *P* < 0.001).

In a second experiment we tested the response of 30 *D. laeve* individuals on the mucus of stressed conspecifics. The same first experimental set-up was used as in experiment 1, except that mucus from stressed conspecifics was used. Mucus from stressed individuals was obtained by simulating the attack of beetles which grasp slugs from behind after following the pedal mucous trail.⁸ This was done by touching the posterior part of three slugs with pincers, without breaking the skin, a few times over 1 min. Such a stimulation changes the amount and composition of the mucus.⁹ After a few minutes the damp paper towel and poplar leaves were transferred to the experimental box. Nineteen out of the 30 individuals almost immediately left the plastic container, despite the copperfoil. Eight other individuals were situated on the area without mucus and three individuals were situated on the area with mucus from stressed individuals. When the individuals that left the box were assigned to the group that did not prefer the area with mucus from stressed conspecifics, the difference in preference was highly significant ($\chi^2 = 19.33$; *df* = 1; *P* < 0.001). The experiment was repeated with the mucus of one stressed individual. In this case, no preference could be detected ($\chi^2 = 0.86$; *df* = 1; *P* = 0.35). Yet, the time spent on areas without mucus (37.77 ± 16 min) was significantly higher than the time spent on areas with the mucus of the single stressed individual (20.10 ± 16.13 min; paired *t*-test: -3.02; *df* = 29; *P* < 0.001; Fig. 2B). Animals were more active on areas without mucus (94.75 ± 20.56 %) than on areas with mucus of a stressed conspecific (78.08 ± 31.89%; paired *t*-test: *t* = 2.30; *df* = 24; *P* = 0.03; Fig. 3B).

The difference in response of *D. laeve* on the mucus of con- and heterospecifics was tested using the two experimental set-ups of the first experiment. *D. reticulatum* or *D. panormitanum* individuals were used to obtain heterospecific mucus trails. The results with

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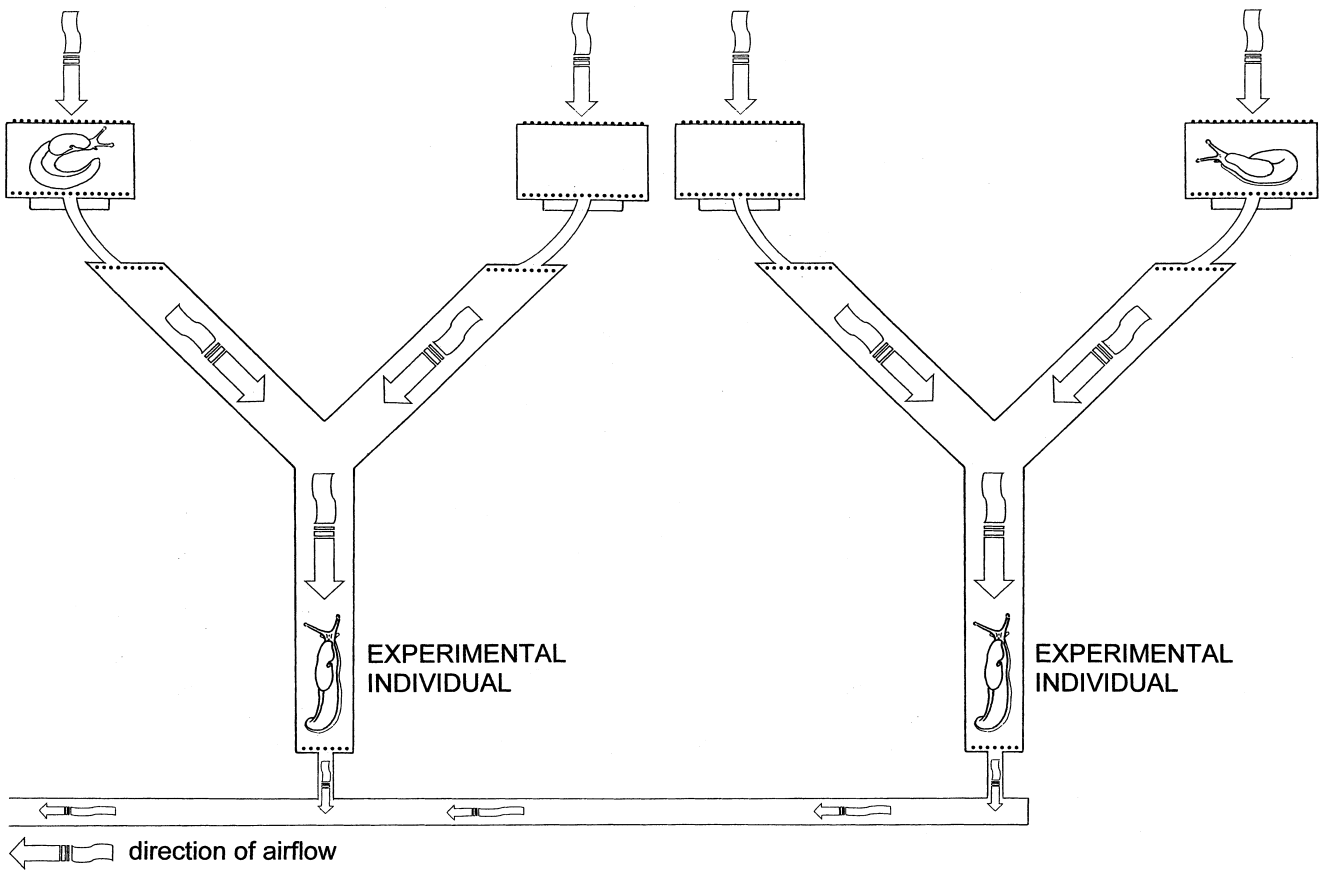


Figure 1. Experimental apparatus for the examination of the response of *D. laevis* on mucus trails and air-borne odours of con- or heterospecific individuals.

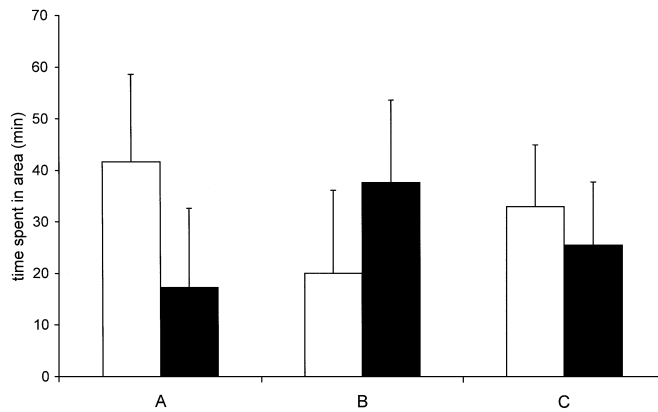


Figure 2. Mean time spent (and standard deviations) in each of the areas of the experimental boxes. A. Mean time spent on area with mucus of conspecifics (white bar) versus mean time spent on area without mucus (black bar). B. Mean time spent on area with mucus of one stressed conspecific (white bar) versus mean time spent on area without mucus. C. Mean time spent on area with mucus from conspecifics (white bar) versus mean time spent on area with mucus of heterospecifics (black bar).

D. reticulatum as heterospecific species did not differ significantly from the results with *D. panormitanum* as heterospecific species (results not shown). Therefore, the results obtained with the two heterospecific species were pooled to obtain a larger sample size. *D. laevis* showed no preference for either of the two areas ($\chi^2 = 0.00$; $df = 1$; $P = 1.00$). The time spent on the area with heterospecific mucus was not significantly different from the time spent on the area with conspecific mucus (33.10 ± 12 min *v.* 25.62 ± 12.27 min.; paired *t*-test: $t = 1.71$; $df = 29$; $P = 0.10$), but there was a

tendency for activity to be higher on the area with conspecific mucus ($74.37 \pm 27.94\%$ *v.* $86.76 \pm 24.20\%$; paired *t*-test: $t = -1.93$; $df = 27$; $P = 0.06$; Figs 2C and 3C). The experiments with the Y-maze showed no preference for either arm ($\chi^2 = 2.13$; $df = 1$; $P = 0.14$).

In a last experiment the response of *D. laevis* on air-borne odours of conspecifics was tested using the experimental apparatus shown in Figure 1. Each of the glass arms of the Y-maze was covered with damp paper towels and poplar leaves. At the end of

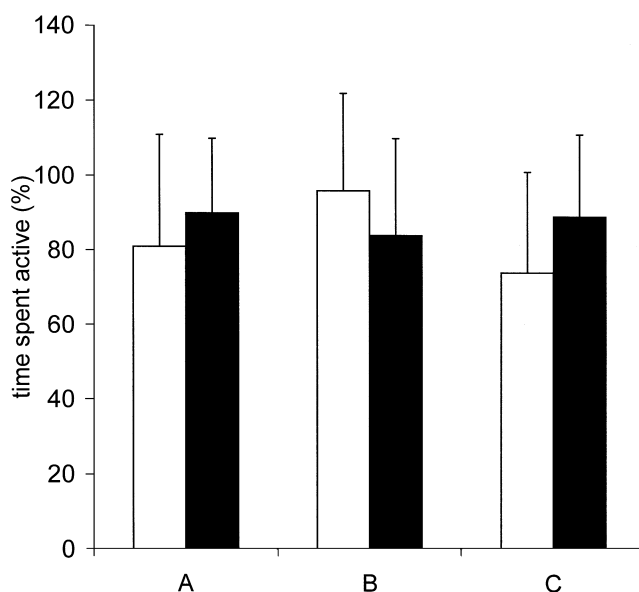


Figure 3. Mean percentage of time actively moving (and standard deviations) in each of the areas of the experimental boxes. **A.** Mean activity on area with mucus of conspecifics (white bar) versus mean activity on area without mucus (black bar). **B.** Mean activity on area with mucus of one stressed conspecific (white bar) versus mean activity on area without mucus. **C.** Mean activity on area with mucus from conspecifics (white bar) versus mean activity on area with mucus of heterospecifics (black bar).

each arm we alternately attached a small plastic tube with and without a conspecific animal. The stem of each Y-maze was attached to a pump to create an air-flow through the Y-maze from the end-tubes towards the experimental animal. One experimental animal was placed in the stem of the Y-maze and we recorded which of the two arms was chosen. Slugs that did not move up into one of the two arms after 30 min were excluded from the analysis. Even low air-flows (<0.05 m/s) resulted in a strong negative response of the test individuals: animals became inactive or tried to move in the direction of the air-flow. Therefore, the experiment was repeated without an air-flow. There was no difference in the preference for one of both arms ($\chi^2 = 0.29$; $df = 1$; $P = 0.59$).

Our results show that the terrestrial slug *Deroceras laeve* prefers areas with mucus of conspecifics to areas without mucus. An experiment with glass Y-maze tubes confirmed the attractive nature of conspecific mucus in *D. laeve*. Further, mucus released by stressed conspecifics had a significant repellent effect on *D. laeve*. Pakarinen¹⁰ showed the same response on mucus from stressed conspecifics in *D. reticulatum*, and explained this behaviour as an adaptation against predation or aggressive competitors. Rollo & Wellington¹¹ investigated intra- and inter-specific agonistic behaviour among several terrestrial slugs. They reported *D. caruane* (Pollonera; = *D. panormitanum*), and *D. laeve* as very aggressive and in a high-density population of *D. laeve* many individuals showed severe wounds typical of those resulting from aggressive interactions. Thus, changes in the composition of the mucus of one individual during or after aggressive interactions may also change the behaviour of nearby conspecifics.

Apart from the ecological functions of this behaviour, mucus may also affect growth and reproduction of *D. laeve*. Both field and laboratory studies have shown that there are adverse effects of density on growth, mortality and reproduction, that can not be attributed to food shortage alone.^{4,5,12-16} It is possible that mucus contains inhibitory pheromones that may reduce (feeding) activity and delay growth in members of the same (and related) species.^{5,6}

Deroceras laeve showed no difference in preference between areas with mucus of conspecifics and areas with mucus of related

heterospecifics. Possibly, *D. laeve* is not able to discriminate between mucus of con- and heterospecifics. Mucus of related species may have similar effects on the behaviour and reproduction of an individual as mucus of conspecifics.^{5,6} This may not be the case in less-closely related species,¹⁷ although Baur¹⁴ and Baur & Baur¹⁶ showed that mucus of *Chondrina clienta* (Westerlund) may have a negative effect upon the reproduction of *Balea perversa* (L.).

Deroceras laeve did not respond to air-borne odours of conspecifics. However, we can not rule out possible influences of air-borne odours on the behaviour of *D. laeve* because our experimental set-up may not have been optimal. Because the experiment was performed without an air current that could create an odour gradient, air-borne odours may not have reached the experimental animals. Moreover, air-borne odours probably were also present in the experiments with the plastic containers. This may explain why activity on areas with and without mucus of conspecifics was the same. In the same way, air-borne odours from mucus of stressed individuals may have resulted in a higher activity on areas without mucus. Activity on areas with mucus from one stressed individual was comparable to activity on areas without mucus of experiment 1, so that it seems unlikely that mucus from stressed individuals decreased activity on that area.

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Growth and reproduction in Hawaiian succineid land snails

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The native land snail fauna of the Hawaiian Islands is one of the most threatened snail faunas in the world;¹ a large proportion of the species, perhaps as many as 90%, may now be extinct.^{2,3} The impacts of introduced predators [e.g. rats and the predatory snail *Euglandina rosea* (Férussac)] and other human-related impacts (e.g. habitat destruction, collecting by shell collectors), superimposed upon long pre-reproductive life and low fecundity (as in achatinelline tree snails),^{4–7} have been implicated in the demise of many of the species. Certain native snails, however, have remained relatively abundant. Among these are some of the rainforest succineids.

Aside from the Achatinellinae,^{4–7} little is known of the life histories of most Hawaiian land snails. However, just as knowledge of life-history characteristics has been important in understanding the high extinction rate of the achatinelline tree snails, such information may also be key to understanding why some succineid species remain abundant in the face of similar pressures.

In this study, the oviparous rainforest succineids *Succinea thaenumi* Ancey and *Catinella rotundata* (Gould), which lay gelatinous, translucent egg masses, usually on aboveground vegetation, were raised in captivity to investigate a range of life-history characteristics.

Three recently hatched juvenile *Succinea thaenumi* and one egg mass from Oloa, Island of Hawaii, were brought into a captive-rearing facility on 3 November 1999. The juveniles were assumed to have hatched very recently because their bodies appeared white through the transparent shells and their digestive glands were not dark, indicating that they probably had not yet fed. Fifteen *Catinella rotundata* from Makaleha, Waianae Mountains, Island of Oahu, were brought into the facility on 30 March 2000.

Snails and egg masses were maintained as colonies in plastic containers (10 cm wide, 10 cm deep, 16 cm tall, with mesh tops; one for each species) subject to night (12 h) and day (12 h) light cycles, and intermittent spraying with water to simulate rain (3 min, every 8 h) in an environmental chamber (16°C at night, 20°C during the daytime), as used for achatinelline tree snail breeding.⁸ The natural diet of these succineids is poorly known, but probably includes fungal material obtained from leaf surfaces.⁹ Each container was provisioned with fresh leaves and branches of the native Hawaiian tree *Metrosideros polymorpha* (Myrtaceae), partly covered with fungus, plus supplemental cul-

tured fungus fortified with a trace of calcium carbonate.⁸ The *M. polymorpha* and fungus were replaced approximately every 14 days when the containers were cleaned. At that time, maximum shell dimension (length) of adults and offspring, number of births and deaths, and number of eggs laid were recorded.

Reproductive characteristics of *Succinea thaenumi* are summarized in Table 1. Average shell length of the three juveniles (termed Cohort A) on 10 November 1999 (7 days after they were collected) was 1.8 mm. The mean maximum length attained by these three individuals was 9.3 mm, on 15 March 2000, after a period of 134 days in captivity. The first egg mass laid by them was found on 1 March 2000, when their average length was 8.8 mm, about 4 months after they had been brought into captivity (as juveniles). Additionally, two of them were observed mating (by 'shell-mounting'¹⁰) on 15 March 2000 (shell lengths 9.8 and 10.1 mm). One was found dead on 30 March 2000, the other two on 28 April 2000, giving a life span of at least 178 days for these latter two snails.

The first *S. thaenumi* hatched from the wild-collected egg mass (Cohort B) was observed on 22 December 1999. Because the previous observation date was 8 December 1999, the snail could have hatched any time during this period and the time to hatching was, therefore, at least 36–50 days.

The average shell length of juveniles that were first seen on 28 April 2000 was 0.85 mm ($n = 15$) and for those first seen on 9 May 2000 was 0.90 mm ($n = 26$). Data from the two hatchings were combined after 9 May 2000 as Cohort C. They reached a mean maximum length of 7.8 mm on 13 October 2000, approximately 5 months from birth.

The first reproductive event witnessed for Cohort C snails was a mating on 4 August 2000. Shell lengths of these snails were 7.0 and 7.7 mm, when the average length of Cohort C snails was 6.0 ± 1.1 mm (mean and SD; $n = 11$). Time to reproductive maturity is therefore approx. 3 months. The first egg mass laid by Cohort C snails was found on 1 September 2000, when the snails were about four months old.

The minimum maximal lifespan for *Succinea thaenumi*, calculated for Cohort C from the earliest possible birth date (13 April 2000) and the date of death of the last Cohort C snails (22 December 2000), was 253 days.

Reproductive characteristics of *Catinella rotundata* are also summarized in Table 1. All wild collected individuals except the largest died soon after coming into captivity, most before reach-