Testing hypotheses of adaptive variation in cricket ovipositor lengths

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Abstract. We experimentally tested a series of hypotheses proposed by Masaki (1979, 1986) for the evolution of ovipositor length in crickets. Female crickets use the ovipositor to bury eggs in the soil, where it was hypothesized to protect their eggs from desiccation, cold and other disturbance. However, we found no effect of depth on the overwinter survival of eggs of three species of Nemobiinae. The probability of hatchlings reaching the soil surface was negatively correlated with depth documenting a significant cost to females laying eggs deep in the soil. Hatchling survival may be an important agent of selection on ovipositor length in habitats of different soil moistures. Hatchling survival in the soil was also correlated with body size, which may impose a constraint on egg-size fecundity trade-offs. Females of a bivoltine population of Allonemobius socius lay eggs at shallower depths when reared under summer compared to fall conditions and, therefore, may be able to respond to selection through behavioral plasticity when morphological adaptation is constrained by allometry.

Key words: Crickets – Ovipositor length – Egg survival – Ovipositioning behaviour

Masaki (1979, 1986) has proposed a variety of adaptive hypotheses to explain both interspecific and interpopulational variation in the length of the ovipositor of crickets. The ovipositor is a needle-like structure that is used to insert eggs into the soil, or in some cases plant stems. The length of the ovipositor is then the maximum depth at which a female can deposit her eggs. Masaki argues that observed ovipositor lengths are the result of a selective balance between the costs and benefits of this structure. The benefits of longer ovipositors are hypothesized to be due to the protection greater depth in the soil provides to the incubating egg from heat, desiccation, extreme cold and predatory losses. The proposed costs arise because a lengthy ovipositor is more costly to produce, and may be a hindrance to movement for the adult female (Masaki 1986). In addition, hatchling survival in the soil may be negatively affected by depth because newly hatched nymphs may experience difficulty digging their way to the soil surface.

Masaki (1979, 1986) has assembled comparative evidence in support of some of these hypotheses. In both Japanese and North American Nemobiinae, he found a positive correlation between the residuals of the allometric relationship between ovipositor length and body size and the dryness of the habitat that is consistent with the hypothesis that greater burial depths protect eggs from desiccation. For two Japanese species, ovipositor residuals also increase along the cline of severity of winter, supporting the hypothesis that eggs are insulated from extreme cold by the soil (Masaki 1979). Mousseau (1988) has found a similar pattern in the North American species Allonemobius fasciatus and A. socius. Masaki (1986) also notes that in sister species ovipositors tend to be longer in the egg-overwintering species than those that overwinter as nymphs and lay non-diapause eggs during summer. This is further corroborative support for the protection hypothesis because eggs in diapause must remain in the soil for six months or more, compared to only 2–3 weeks for species laying direct-developing eggs.

In this paper we experimentally test some of Masaki’s hypotheses with five cricket species that differ in habitat and ovipositor length. We ask whether there is an effect of egg depth on overwintering survival, and if hatchling survival in the soil is affected by egg depth and soil moisture.

Masaki (1986) notes an interesting conflict by the selection imposed on ovipositor length in bivoltine egg-diapausing species; for first generation females which lay direct-developing eggs in mid-summer, shorter ovipositors should be favoured, while longer ones will be selected for in the second generation because the eggs that are laid will overwinter in diapause. This type of selection could lead to the evolution of phenotypic plasticity in ovipositor length, or perhaps plasticity in egg-laying behaviour. We test these hypotheses, predicting that females in a fall environment should have longer ovipositors and should lay their eggs deeper in the soil than females reproducing in summer conditions.
Materials and methods

Overwintering survival

In this experiment eggs were placed outside for the winter to determine if overwintering survival was affected by egg depth. Adults of *Allonemobius allardi*, *A. fasciatus* and *Eumomobius carolinus* were collected from the Montreal region in September 1988 and reared at 25°C, 12:12 LD in the laboratory (see Bradford and Roff [11 press] for details of rearing methods). All three species are univoltine in this region. *A. allardi* inhabits dry fields, *A. fasciatus* is found in moist fields and *E. carolinus* prefers extremely wet areas along water bodies (Vickery and Johnstone 1973). Morphological measurements for the three species are given in Table 1. Eggs were collected from each species and separated into groups of 30 on moistened cheesecloth squares, incubated for 20 days at 25°C and then transferred to a 4°C refrigerator. In mid-November the eggs were transferred to disposable 10 ml plastic beakers. Moistened soil was added to the beakers to a depth of 4, 8 or 12 mm from the top; the soil was packed gently with a 15 g weight. The cheesecloth with eggs was placed on the soil, and the beaker was then filled to the top with soil and lightly packed. In this experiment the bottoms of the beakers were replaced with mosquito netting to allow the free passage of precipitation through the beaker. The beakers were inserted in the soil at two sites in the Montreal region; the first was a moist field where *A. fasciatus* was common, the other was a more gravelly hillside where *A. allardi* was present. The soil used in the beakers was collected from each of the sites. No adult crickets were present when the eggs were transplanted in the field that would contaminate the beakers with additional eggs. There were 5 replicates per species/depth/site treatment.

In early May 1989 the beakers were retrieved from the field and rewarmed gradually to 25°C. The contents of each beaker were spread onto small petri dishes and moistened; they were checked daily and the number of hatchlings appearing recorded. By spreading out the soil we attempted to eliminate the effects of depth on hatching survival, so that we could isolate the effect of depth on the survival of the eggs only.

Hatchling survival

Two experiments were conducted to estimate the effect of depth on the survival of hatchlings in the soil. In the first trial, adults from a partially bivoltine population of *A. socius* from Danville, VA, USA (36°40'N, 79°25'W) were reproduced under diapause-averting conditions. Eggs were collected, plated on paper towels and incubated at 30°C until the embryo was clearly visible inside the chorion in most of the eggs (i.e., they were 2–3 days away from hatching). The eggs were then counted out onto small cheesecloth squares in groups of 25. The eggs were transferred to the plastic beakers filled to depths of either 3, 6 or 9 mm from the top; the soil was packed as before. Three soil moisture levels were used, 15, 25 and 35% water, by weight. There were 6 replicates per depth by moisture combination.

The beakers were placed individually in plastic sandwich boxes with a petri dish filled with plaster of Paris, when moistened, served to maintain the humidity in the box. The boxes were placed in a 30°C incubator, and were checked daily; emerged hatchlings were counted and removed.

The second trial used eggs collected from our *A. fasciatus*, *A. allardi* and *E. carolinus* populations from the Montreal region and eggs from a *Gryllus firmus* stock originating from Florida (Roff 1986). Diapause eggs from the three Montreal area species were placed in a 4°C incubator for 4 months and then were incubated at 25°C until just prior to hatching. Eggs from *G. firmus* are all non-diapause; they were incubated at 30°C for 10 days before transfer to 25°C. The same procedure as the first trial was followed, except eggs were placed at 4, 8 and 12 mm, and a single soil moisture of 20% was used. *G. firmus* eggs were also placed at 16 mm depth. There were 5 replicates per treatment.

Behaviour experiments

Two experiments were conducted to test the hypothesis of seasonal variation in oviposition behaviour. In the first experiment *A. socius* nymphs from Danville, VA, population were hatched from diapausing and reared at 28°C, 14:10 LD for 20 days. The nymphs were kept in plastic sandwich boxes, and fed lettuce and crushed cat food (Mousseau and Roff 1989). At day 20, the nymphs were divided into two groups, and placed in incubators at either 30°C, 15:9 LD (summer conditions), or 25°C, 13:11 LD (fall conditions). At eclosion, adults were transferred to larger plastic mouse boxes (30 × 17 × 14 cm), but kept under the same environmental conditions. There were 7 cages in each environment, each containing 5–7 females and 5–7 males.

To estimate the depth at which eggs were laid we provided each cage with a device consisting of a stack of 35 mm slide holders, with cheesecloth rectangles filling the center hole. The slide holders were 1.5 mm thick. A layer of mosquito netting was placed between each slide. The cheesecloth was moistened with 10 ml of water. Females were allowed to oviposit in the device for 48 h, before it was changed. After removal from the cage the mosquito netting was used to separate the layers of cheesecloth, and eggs laid in the cheesecloth were removed and counted. Three replicates were taken over time from each box.

The second experiment was designed to separate the effects of the nymph and adult rearing environments on the egg laying behaviour. Newly hatched nymphs were raised as before, but all were kept at 30°C, 15:9 LD until eclosion. Adults were reared at these conditions for 10 more days to ensure sexual maturity, and were split into 4 cages of 8–10 each of females and males. An egg laying device was added to each cage; two cages were transferred to 25°C, 13:11 LD, and two were kept at 30°C, 15:9 LD. After 5 or 7 days (depending on weekends) the adult cages were switched to the other environment, and the devices were introduced for a second 7 days.

The oviposition devices were modified slightly in the second experiment. Moistened sand (35% water by volume) was added to the stack of slides instead of cheesecloth. A thin knifeblade was used to separate the slides and the sand contained within them, the eggs were separated in water and counted. The devices were changed every day, except for 2-day intervals on weekends.

Table 1. Mean lengths of adult female femurs, ovipositors, freshly laid eggs and hatching femurs (all in mm, based on samples of 10–15, except for *G. firmus* eggs where *N = 98*) for 4 cricket species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Adult femur</th>
<th>Ovipositor</th>
<th>Egg length</th>
<th>Hatching</th>
<th>E/F</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. firmus</em></td>
<td>13.05 ± 0.24a</td>
<td>17.15 ± 0.31a</td>
<td>2.77 ± 0.02a</td>
<td>1.29 ± 0.008a</td>
<td>0.21</td>
</tr>
<tr>
<td><em>A. allardi</em></td>
<td>6.53 ± 0.09b</td>
<td>8.65 ± 0.22b</td>
<td>1.91 ± 0.02b</td>
<td>1.02 ± 0.012b</td>
<td>0.29</td>
</tr>
<tr>
<td><em>A. fasciatus</em></td>
<td>6.25 ± 0.08c</td>
<td>7.33 ± 0.14c</td>
<td>1.83 ± 0.02c</td>
<td>0.91 ± 0.014c</td>
<td>0.29</td>
</tr>
<tr>
<td><em>E. carolinus</em></td>
<td>4.68 ± 0.10d</td>
<td>2.84 ± 0.04d</td>
<td>1.75 ± 0.01d</td>
<td>0.88 ± 0.009d</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Shown are means with SE; significant differences are indicated by different letters (α = 0.05, REGWF test, SAS 1988). E/F is the ratio of egg to adult femur length.