

Geographic Variation in Size and Oviposition Depths of *Romalea microptera* (Orthoptera: Acrididae) Is Associated With Different Soil Conditions

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ABSTRACT Intraspecific geographic variation in morphology and behavior can be indicative of populations that are adapted to local environmental conditions. Heterogeneity in the abiotic environment, such as soil conditions, can be an important driver of local adaptation. In south Florida, mean body sizes in populations of the lubber grasshopper *Romalea microptera* (Beauvois) (Orthoptera: Acrididae), increase from west to east. We tested how body size variation, population origin, and soil physical characteristics are associated with oviposition depth in the soil. In a laboratory experiment with homogeneous soil conditions, size-corrected oviposition depth (analysis of covariance) differed among three populations: females from a western population oviposited at the shallowest depths; females from an eastern population oviposited the deepest; and a central population oviposited at intermediate depths. A similar pattern also was observed in two field experiments conducted on two additional populations not used in the laboratory experiments. In addition, size-corrected oviposition depth in the field experiments declined with increasing soil compaction for both populations but the decline was significantly steeper for the eastern population. High soil moisture yielded significantly shallower size-corrected oviposition depth only for the eastern population. Female size was positively related to oviposition depth in both field experiments, but the relationship between female size and oviposition depth did not differ among populations (i.e., equal slopes). We suggest that local soil conditions might be an important selection pressure and behavioral cue in determining oviposition depth. Choice of vertical position of the egg pod within the soil is discussed as one possible mechanism by which females can influence offspring viability.

KEY WORDS behavioral choice, Florida, local adaptation, oviposition choice, phenotypic variation

Intraspecific geographic variation in phenotype is well documented in many species of insects (Masaki 1979, Mosseau and Roff 1989, Huey et al. 2000). Geographic clines are ideal systems in which to investigate how and why phenotypic variation arises. Phenotypic variation along a cline can arise because natural selection favors different phenotypes in different environments. Environmental variation (e.g., temperature, precipitation) is often structured along geographic axes (e.g., north–south). Spatial variation in abiotic conditions can select for phenotypic clines along those same gradients. For example, insect body size often shows clinal variation with latitude or altitude, with average body size increasing with increasing latitude (or altitude) because large size is adaptive for living in the colder temperatures found high latitudes (or al-

titudes; Nylin and Svard 1991, Tefler and Hassall 1991, Johnston and Bennett 1996). Although latitudinal and altitudinal gradients are the most frequently studied clines, environmental variation, and thus phenotypic variation, can be structured along longitudinal axes as well (Davidson 1977, Meiri et al. 2005).

Soil conditions (e.g., moisture, compaction) vary across the landscape. For soil-ovipositing species, spatial variation in soil conditions could select for inter-population differences in oviposition depth. For species that oviposit in soil, selection should shape appropriate morphological adaptations suited for digging (e.g., body size, ovipositor length; Uvarov 1977, Mousseau 2000), sensory mechanisms to assess the soil conditions (Isley 1938; Choudhuri 1956, 1958; Uvarov 1977; Mousseau 2000), and behavioral mechanisms to produce flexible responses to soil conditions based on morphological capacity. Female body size and shape (i.e., physical capacity) and oviposition behavior (based on sensory feedback) are two potential targets of selection to optimize oviposition depth for a given set of soil conditions. If soil conditions vary spatially and select for body size and oviposition behavior, then

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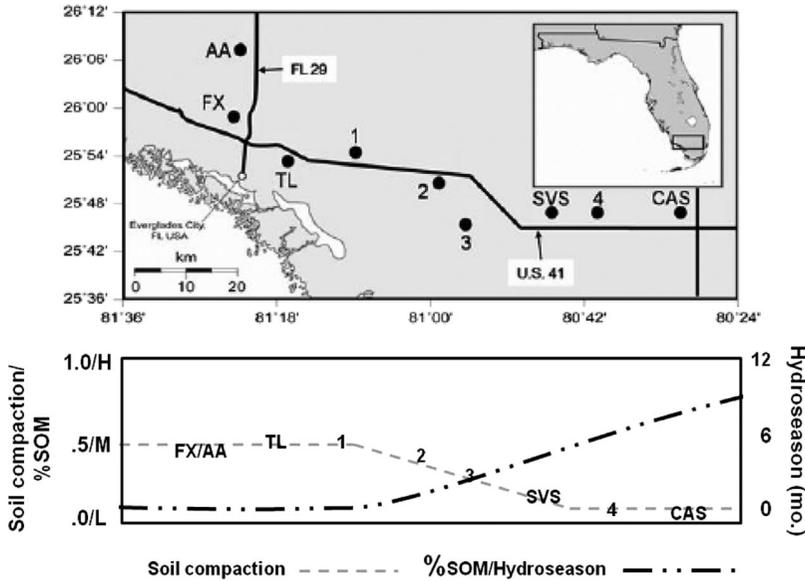


Fig. 1. Field sites and compaction sampling sites along a west–east transect in south Florida. Sites with populations used for these experiments are labeled with a two- or three-letter abbreviation; those sites where only soil compaction was measured (i.e., no animals tested for oviposition depth) are numerically labeled. Three interrelated environmental variables potentially influence *R. microptera* oviposition depth across the south Florida landscape: soil compaction (dashed line; 0.0, 0.5 and 1.0 kg/cm²), percentage of soil organic matter (% SOM; dashed-and-dotted line; L, low; M, medium; H, high), and typical hydroseason (dashed-and-dotted line; 0, 6, and 12 mo). Soil compaction is based on empirical data and reflects the mean compaction value for all depths, 0, 5, and 10 cm (see Table 1); % SOM and hydroseason curves are based on qualitative descriptions from geology literature of south Florida. Hydroseason determines % SOM. A longer hydroseason reduces aerobic breakdown of plant matter resulting in peat soils. A short hydroseason builds marl soils. As % SOM increases, soil compaction is reduced (Lodge 2004).

a cline in body size could arise from continuous geographic variation in soil conditions. In terms of physical capacity, evidence indicates that large body size, greater ovipositor length, and longer abdominal stretching in soil-ovipositing species are positively correlated with depth of egg pod deposition (Zimin 1938, Uvarov 1966, Masaki 1979, Braker 1989, Mousseau and Roff 1995). Previous research suggests that the optimal oviposition depth is a balance between the benefits of increased egg and hatchling survival and the costs of deep oviposition (e.g., female time, energy, and exposure to enemies; hatchling's ability to excavate from deeply laid eggs) and imply females should choose depths that optimize this trade-off (Bradford et al. 1993, Mousseau 2000, Branson and Vermeire 2007).

In this study, we examine the interpopulation differences in oviposition depth among south Florida populations of the lubber grasshopper *Romalea microptera* (Beauvois) (Orthoptera: Acrididae). The average adult body size of lubbers exhibits a geographic cline increasing longitudinally from west to east among populations in south Florida (Huizinga et al. 2008, Jannot et al. 2009). Female size probably limits maximum oviposition depth. Because female size varies among populations, we predicted that oviposition depth would also vary among populations. In addition, soil conditions (e.g., moisture, compaction) also vary from west to east along the same transect in south

Florida, suggesting a link between soil conditions, oviposition depth, and adult grasshopper body size. Hydroperiod, which has a strong effect on soil conditions, varies from short hydroperiods in the west to longer hydroperiods in the east (Fig. 1; also see Lodge 2004). Short hydroperiods (2–6 mo annually) found in the west form marl soils (variable composites of clay, silt, and calcium carbonate) (Fig. 1; Ewel 1990, Snyder et al. 1990, Lodge 2004), whereas long hydroperiods (9+ months) that occur at the eastern sites result in peat soils (built-up layers of decomposing vegetation) (Fig. 1; Kushlan 1990, Gleason and Stone 1994, Gunderson and Snyder 1994, Lodge 2004). Marl is drier and more compacted than peat; therefore, soil moisture should increase from west to east and soil compaction should decrease along the same gradient.

We predicted that optimal oviposition depth should depend on soil conditions. Specifically, if soil moisture and soil compaction vary geographically and natural selection has selected for optimal oviposition depth, then populations should oviposit at depths that reflect the optimal depth for the soil conditions at their site of origin, irrespective of adult female body size. This result would be consistent with the hypothesis of local adaptation of oviposition choice to soil conditions. Alternatively, oviposition depth might be limited by female body size and not a function of soil conditions. This postulate predicts that individual body size limits the depth of oviposition and females always oviposit at

Table 1. Soil compaction means (kg/cm² ± 1 SE, N = 11) for three depths and *R. microptera* female size (mean pronotum length, mm ± 1 SE) at nine locations along the geographic cline (see Fig. 1)

Location	Region	Compaction			Female size	N (females)
		0 cm	5 cm	10 cm		
FX	Western	0.32 (0.04)	0.42 (0.04)	0.55 (0.06)	17.3 (0.1)	142
AA	Western	0.22 (0.04)	0.40 (0.05)	0.40 (0.05)	17.2 (0.1) ^a	308
TL	Western	0.34 (0.03)	0.52 (0.04)	0.64 (0.03)	17.8 (0.2) ^a	66
1	Central	0.27 (0.04)	0.55 (0.05)	0.64 (0.04)	NA	
2	Central	0.28 (0.04)	0.38 (0.04)	0.57 (0.04)	18.6 (0.3) ^a	19
3	Central	0.25 (0.05)	0.35 (0.04)	0.37 (0.03)	18.5 (0.4) ^a	43
SVS	Eastern	0.13 (0.02)	0.15 (0.02)	0.14 (0.04)	19.7 (0.2) ^a	95
4	Eastern	<0.1	<0.1	<0.1	19.5 (0.4) ^a	22
CAS	Eastern	<0.1	<0.1	<0.1	20.3 (0.2)	141

Locations with populations used for these experiments are labeled with a two- or three-letter abbreviation; all other locations sampled are numerically labeled. NA, not applicable.

^a Data from Jannot et al. (2009).

the maximum depth possible because the maximum depth yields the highest offspring survival. In a laboratory study and two field experiments, we examine three variables and their relationship to oviposition depth: 1) the size of the female; 2) the population origin of the female; and 3) soil characteristics (type, moisture, and compaction). We also present data on variation in soil characteristics experienced by females in the wild.

Materials and Methods

Study Site and Species. We studied oviposition depth of *R. microptera* populations in south Florida along a 120-km west-to-east transect in and around the greater Everglades ecosystem (Fakahatchee State Park, Big Cypress National Preserve, Everglades National Park; Fig. 1). Altitude in south Florida varies by only a few meters, but elevation changes of less than one meter can produce significant differences in hydrology, soil composition, and plant community (Lodge 2004). The west–east transect is characterized by marl soils, relatively short hydroperiods, and marl prairies in the west (e.g., AA, FX in Fig. 1) gradually shifting toward peat soils, longer hydroperiods, and sawgrass prairies in the east (e.g., SVS, CAS in Fig. 1). Characteristic host plants common to our field sites include the following : *Phragmites australis* (Cav.) Trin. ex Steud. (Cyperales: Poaceae), *Crinum americanum* L. (Liliales: Liliaceae), *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult. f. (Arecales: Arecaceae), *Cladium mariscus* (L.) Pohl ssp. *jamaicensis* (Crantz) Kük. (Cyperales: Cyperaceae), *Salix caroliniana* Michx. (Salicales: Salicaceae), *Baccharis halimifolia* L. (Asterales: Asteraceae), and *Schinus terebinthifolius* Raddi (Sapindales: Anacardiaceae) (K. Kocot and J.E.J., unpublished data). Linear sizes of adult lubbers increase by 30% from west to east along this transect (Huizinga et al. 2008, Jannot et al. 2009). Average adult female body sizes (pronotum length) for populations can be found in Table 1. Lubbers are large, flightless, polyphagous herbivores (Rehn and Grant 1961, Whitman and Orsak 1985). In south Florida, *R. microptera*, oviposition begins in late May and lasts into August (J.E.J., personal observations), and females can lay

one to three egg pods (Walker et al. 1999, Mefferd et al. 2005). Adult females oviposit in soil by digging a hole with short ovipositor valves at the tip of their extensible abdomen. A female uses her abdomen to dig into the soil; egg pods are placed vertically within the hole and subsequently capped with a foam plug. Females spend, on average, ≈80 min (±22 min) ovipositing in the wild (Stauffer and Whitman 2007).

Soil Compaction in the Field. We measured soil compaction at twelve sites along the longitudinal transect, including sites at or near the populations used in this study (see Fig. 1; three sites in the western region are outside the frame of the figure). At each site, a 100-m transect was laid out perpendicular to the access road starting ≈20 m from the road to minimize road influence. At 10-m intervals along each transect, soil compaction (=force required to penetrate the soil; kilograms per square centimeter) was taken with a soil penetrometer (Pocket Penetrometer, Forestry Suppliers, Inc., Jackson, MS) at 0-, 5-, and 10-cm soil depth. These depths were chosen because they span the range of observed *R. microptera* oviposition depths in the laboratory study (see Results).

Laboratory Experiment. Females were obtained by collecting fifth instar (=final) nymphs from three field sites (western, AA; central, TL; eastern, SVS; see Fig. 1) during May 2007. Females were transported to Illinois State University, housed in individual Tupperware containers with screen lids, and placed in environmental chambers, with a photoperiod of 14:10 (L:D) h and a 32:24°C (day:night) daily temperature cycle. Lubbers were given romaine lettuce, rolled oats, bran flakes, and water (≈15 ml) ad libitum throughout the experiment. Cages were cleaned and new food and water added every 1–2 d for the duration of the experiment. Pronotum length of each adult female was determined ≈24 h after molting to the adult stage (final N postmortality: AA, 6; TL, 16; SVS, 6). When females exhibited signs of oviposition (i.e., distended abdomen; digging motion with ovipositor valves), females were placed inside individual (17.8- by 27.9-cm) clear, ovoid plastic containers filled to a depth of 21 cm with dry, white sand as a standard oviposition substrate. Sand was used to provide a uniform, standardized, easily available substrate that is

similar to the sandy soils in south Florida, and which was known from other experiments, would stimulate grasshopper oviposition behavior. The depth of oviposition for each egg pod was measured (centimeters) from the surface of the sand to the bottom of the egg pod.

Field Experiments. To determine whether populations exhibit variation in oviposition depth in response to soil characteristics in the field, we conducted two seminatural experiments with two field populations. During June 2008, 161 western (FX) and 221 eastern (CAS) adult female *R. microptera* were collected and measured for pronotum length. Collections were staggered by ≈ 2 wk to account for differences in ages among the populations. The timing of the collections also ensured that all females were captured before their first oviposition event. Females were housed in a location central to the field sites in group field cages (≈ 40 by 25 by 20 cm) at equal densities, kept separate by population origin, and measured for pronotum length. Females had access to both sun and shade to allow seminatural thermoregulatory behavior. Each cage received an ad libitum supply of romaine lettuce and rolled oats, which was replaced during daily cage cleaning. Females exhibiting abdominal distension and digging behavior were placed individually on soil from one randomly chosen experimental treatment (see below) until oviposition was completed (≈ 40 –120 min.). Each female oviposited once, then was removed from the container and released back to her original site alive and unharmed. We measured the depth from the soil surface to the top and bottom of the egg pod.

Field Experiment 1: Soil Type. To determine the effect of soil type on oviposition depth, both populations were subjected to two treatments differing only in soil composition, either marl or peat. Marl soils were collected from the western (FX) site and peat soils were collected from the eastern (CAS) site. For each soil type, the soil mixture in the experiment was a homogenized aggregate of five samples. Soils were hand sifted to break up clumps and to remove large debris (e.g., rocks, roots) and air-dried for 3 d. Treatments were prepared by adding 15 cm of loose soil to round, plastic planting pots (≈ 1.7 liters), wetting it ($\approx 10\%$ moisture by mass), and tamping down to achieve loose compaction (loose < 0.1 kg/cm²) as tested with a soil penetrometer (described above). One female was placed on each pot (=single soil type). Numbers of replicate females were as follows: for western (FX), marl = 20, peat = 18; for eastern (CAS), marl = 20, peat = 20.

Field Experiment 2: Moisture and Compaction. To test the effects of soil moisture and compaction on depth of oviposition in the two populations, we created five treatments that were combinations of moisture and compaction in round, plastic containers (946 ml). We used marl soil because populations showed no differences based on soil type (see Results), and marl soil enabled us to construct consistent moisture and compaction treatments. Three moisture treatments were set up with loose compaction (< 0.1 kg/cm²) by

thoroughly mixing soil and tap water to the appropriate moisture level and then adding 10 cm of the moistened soil to containers. There were three moisture levels (by mass): saturated $\approx 27\%$ moisture; moist $\approx 18\%$ moisture; and dry $\approx 9\%$ moisture. We created three compaction treatments by adding 10 cm of dry soil ($\approx 9\%$ moisture) to containers (946 ml) and tamping soil in progressive layers to maintain consistent compaction throughout the container. There were three levels of compaction: loose < 0.1 kg/cm²; medium = 0.5 kg/cm²; and hard = 1.0 kg/cm². These treatments spanned the range of compaction levels found in the field (Table 1).

Statistical Analyses. Field soil compaction readings were grouped into western, central, and eastern regions by dividing the longitudinal transect into three 40-km sections. These groupings were chosen because we expect the western region to be primarily marl, the eastern region to be primarily peat, and the central region to be varying mixtures of peat and marl. We analyzed compaction at 0, 5, and 10 cm (square root transformed to meet assumptions of normality and homogeneity of variances) with a nested multivariate analysis of variance (MANCOVA), with region as a fixed effect and site within region as a random effect. Contrasts of mean pairwise multivariate differences among regions, with a Bonferroni correction, were used to test for differences between regions.

For the laboratory experiment, we used analysis of covariance (ANCOVA) (PROC GLM, SAS Institute 2003) to examine the effect of site (AA, TL, SVS) and the covariate pronotum length (millimeters) on depth to the bottom of the egg pod. Preliminary tests indicated that slopes of depth versus pronotum was similar for all populations and therefore the pronotum \times site interaction was removed from the final analysis. Pairwise differences among the least-squares means of oviposition depth were compared using the PDIF option of the LSMEANS statement and a Tukey corrected *P* value.

For field experiment 1, depths to the top and bottom of the egg pod were analyzed using a MANCOVA with population, soil type, and population \times soil type as the class effects. Pronotum length was used as the covariate, thus any differences in depth due to population or soil type are independent of female size. For field experiment 2, depths to the top and bottom of the egg pod as a function of moisture and compaction were analyzed using a MANCOVA with population, moisture-compaction treatment, and population \times treatment as the class effects and pronotum length as the covariate, to control for the effects of female size. Preliminary analyses indicated that slopes of multivariate relationships of depth variables to pronotum length were similar for all groups and therefore the pronotum \times treatment interaction was removed from the final analysis. For the moisture-compaction data, pairwise multivariate contrasts of mean oviposition depths were used to test differences between specific treatments. Data for all analyses met assumptions of normality and homogeneity of variances. Analyses

Table 2. Nested MANOVA results for square root transformed field soil compaction readings at 0-, 5-, and 10-cm soil depth for three geographic regions (W, western; C, central; E, eastern) as a function of site nested within region

Source	Wilks' lambda	F	df	P
Region	0.16	3.55	6, 14	0.0239
Site (region)	0.22	8.27	27, 325	<0.0001
Contrasts				
W vs. C	0.95	0.11	3, 7	0.9503
W vs. E	0.17	11.18	3, 7	0.0046
C vs. E	0.23	8.02	3, 7	0.0115

Multivariate pairwise contrasts of regions are reported. Pairwise *P* values are significant at *P* < 0.0167 (Bonferroni correction of 0.05/3).

were conducted with SAS version 9.1 on Windows XP platform (SAS Institute 2003).

Results

Soil Compaction in the Field. Compaction values ranged from <0.1 kg/cm² in the east to >0.5 kg/cm² in the west (Table 1). Although the trend followed the expected pattern based on hydrology in south Florida (Fig. 1), western and central regions were not statistically separable ($F_{3,7} = 0.11$; *P* = 0.95). However, both western ($F_{3,7} = 11.18$; *P* = 0.0046) and central ($F_{3,7} = 8.02$; *P* = 0.0115) regions were significantly more compacted than were eastern sites (Table 2; Supplemental Table 1). Soil compaction seems not to follow a linear gradient along the transect; rather, there is a shift in compaction between the western and eastern sites. Overall, compaction increased with depth (Supplemental Table 1).

Laboratory Experiment. Depth to the bottom of the egg pod differed significantly among populations when sand was presented as a standard oviposition substrate (ANCOVA: site $F_{2,84} = 56.34$; *P* < 0.0001) (Fig. 2). The western population (AA) oviposited at the shallowest depth whereas the eastern population (SVS) oviposited the deepest and the central population (TL) oviposited at an intermediate depth (Fig.

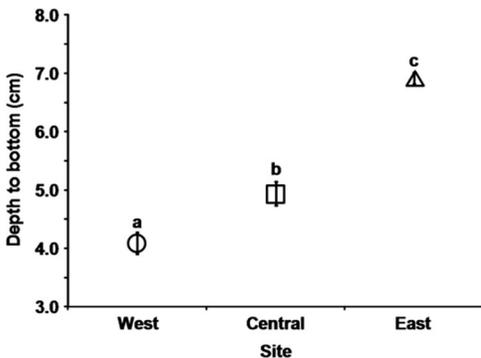


Fig. 2. Oviposition depth (soil surface to the bottom of the egg pod) in the laboratory experiment (least squares mean \pm 1 SE) for *R. microptera* females from three field sites. Different letters indicate differences between sites at $\alpha \leq 0.0004$.

Table 3. MANCOVA results for soil type exp with *R. microptera* on depth to the top and bottom of egg pods

Source	Wilks' lambda	F	df	P	Standardized canonical coefficient	
					Top	Bottom
Pronotum	0.85	6.05	2, 71	0.004	-0.30	1.97
Pop	0.71	14.71	2, 71	<0.0001	-0.30	1.97
Soil	0.96	1.40	2, 71	0.25	-1.10	2.44
Pop \times soil	1.00	0.07	2, 71	0.93	-1.20	2.47

2). Interestingly, this result was independent of female size; depth of oviposition was not significantly related to female size in this experiment (ANCOVA: pronotum length $F_{1,84} = 1.35$; *P* = 0.12).

Field Experiment 1: Soil Type. Depth of oviposition differed between populations and was positively related to pronotum length, but was not affected by soil type or interactions among population, body size, and soil type (Table 3). Depths to top and bottom of egg pods were significantly shallower for the western (FX) than eastern (CAS) females ($F_{2,71} = 14.71$; *P* < 0.0001) (Fig. 3), independent of female pronotum length (i.e., no population by pronotum length interaction). Depth was positively related to pronotum length; however, the lack of a population \times pronotum interaction indicates that the change in oviposition depth as a function of pronotum length did not differ between the two populations (Table 3; Fig. 4). When adjusted for female pronotum length via ANCOVA, animals from the eastern population laid 0.9 cm deeper to the bottom of the egg pod than did western females. Larger body size was associated with deeper oviposition: for each 1 mm of pronotum length, females oviposited \approx 1 mm deeper (Fig. 4).

Field Experiment 2: Moisture and Compaction. Oviposition depths were again positively related to pronotum length, and affected by population, moisture-compaction treatment, and the population \times treatment interaction (Table 4). Depths to top and

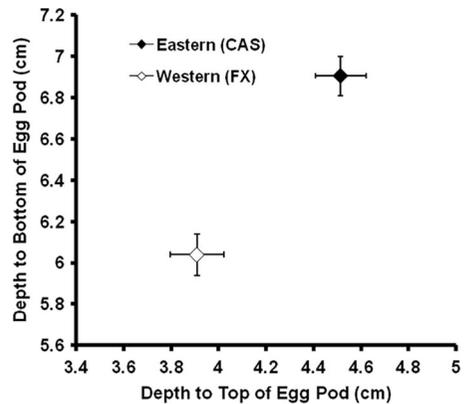


Fig. 3. Oviposition depth (soil surface to the top and bottom of egg pods; least squares mean \pm 1 SE) for *R. microptera* females from eastern sites (solid; CAS) and western sites (open; FX).

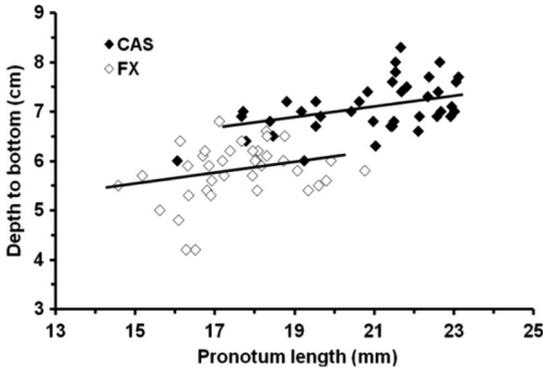


Fig. 4. Oviposition depth (soil surface to the bottom of egg pods) as a function of *R. microptera* female pronotum length for each population in the soil type experiment. Slopes for eastern females (CAS, solid symbols: depth = 0.12 × pronotum length + 4.59) and western females (FX = open symbols: depth = 0.12 × pronotum length + 3.76) are significantly different from zero ($P < 0.01$) but not significantly different from each other ($P > 0.20$).

bottom showed similar responses to pronotum length, population, treatment, and population × treatment interaction; therefore, we only present means for depth to the bottom (Fig. 5). Multivariate pairwise contrasts indicated that, independent of size, eastern (CAS) females laid significantly deeper than western (FX) females for all moisture classes (asterisks, Fig. 5). As moisture increased, there was a significant decrease in size-adjusted oviposition depth for eastern females in saturated soil (small x's and y, Fig. 5). In contrast, western females' size-adjusted oviposition depths did not differ across moisture treatments (capital X's, Fig. 5). For both populations, as compaction increased, size-adjusted depth of oviposition decreased (Fig. 5) but decreased more rapidly for eastern (CAS) females (Fig. 5). Between populations, eastern females oviposited deeper than western females (FX) only under loose soil conditions (asterisk, Fig. 5); oviposition depth did not differ between populations in medium or hard compaction (Fig. 5). Within populations, the difference in the size-adjusted mean oviposition depth between loose and hard compaction was 2.1 cm for eastern females (small a, b, c in Fig. 5) and 1 cm for western females (capital A, B, C in Fig. 5).

Discussion

We found that (1) *R. microptera* females in south Florida exhibit significant interpopulation differences

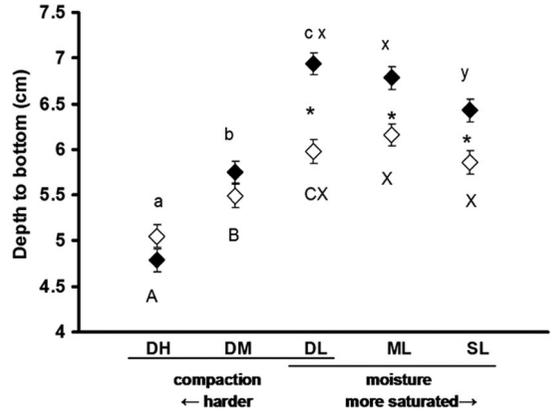


Fig. 5. Oviposition depth (soil surface to bottom of egg pod) by *R. microptera* for five soil moisture and compaction treatments by population. DH, dry, hard; DM, dry, medium; DL, dry, loose; ML, moist, loose; SL, saturated, loose. Significant differences ($P < 0.05$) are indicated by asterisks (*) between populations on the same treatment; small letters for eastern females (solid symbols; CAS) between treatments (a, b, c for compaction; x, y for moisture); and capital letters for western females (open symbols; FX) between treatments (A, B, C for compaction; X for moisture). N for FX: DH = 19, DM = 18, DL = 20, ML = 22, SL = 20. N for CAS: DH = 20, DM = 20, DL = 20, ML = 20, SL = 21.

in oviposition depth: western, small-bodied females oviposit at shallow depths but eastern, large-bodied females oviposit relatively deeper; (2) population differences in oviposition depth are independent of female body size and are expressed in both the field and the laboratory; (3) larger females oviposit deeper than small females; (4) increasing soil compaction decreases oviposition depth in both populations, but the strength of the response was greater among females from an eastern population; (5) increasing soil moisture decreases female oviposition depth in an eastern population but does not significantly change oviposition depth in a western population; and (6) soil compaction is greater at western versus eastern sites, suggesting western females oviposit in compacted soils in the wild. Our results suggest the following points, which we discuss below: 1) oviposition depth is a population-specific flexible response to soil conditions; 2) population-specific responses are consistent with the hypothesis of local adaptation to spatial variation in soil conditions; and 3) despite modest body size-depth correlations, population origin is likely a more important determinant of oviposition depth than

Table 4. MANCOVA results for moisture–compaction experiment with *R. microptera* on depth to the top and bottom of egg pods

Source	Wilks' lambda	F	df	P	Standardized canonical coefficient		
					Canonical variate	Top	Bottom
Pronotum	0.94	5.73	2, 184	0.0038		-0.83	2.28
Pop	0.91	8.78	2, 184	0.0002		0.13	1.54
treatment	0.41	26.16	8, 368	<0.0001	1	0.59	1.10
					2	-2.50	2.55
Pop × treatment	0.86	3.69	8, 368	0.0004	1	-0.57	2.11
					2	2.50	-1.81

female body size. Finally, there is a paucity of information pertaining to the fitness costs and benefits of oviposition depth in soil-ovipositing species. We conclude with a framework for future research on the adaptive significance of oviposition depth.

Oviposition depth in response to a range of soil conditions seems to be more flexible in the eastern population than in the western population. For example, in loose compaction soils, similar to conditions found in the east, the eastern population oviposited significantly deeper than the western females. However, eastern females reduce oviposition depth to a greater extent than western females in response to increases in compaction. Similarly, moisture had little influence on oviposition depth for western females, but eastern females oviposited closer to the surface in saturated soils. These data suggest that, independent of size, females from different populations are either actively choosing different oviposition depths in response to compaction and moisture, or are differentially constrained by these physical properties of soils (e.g., a larger body is less effective at digging and burying its larger diameter abdomen in harder soils). Our results indicate that oviposition depth depends on complex interactions between female behavioral choice, soil conditions, and population origin.

The observed population specific responses to soil compaction and moisture are consistent with the hypothesis that populations are adapted to local soil conditions. As predicted, eastern females oviposited deeper than western females. This result was demonstrated in a common laboratory environment and, in that laboratory experiment, was independent of female size suggesting that populations have become locally adapted to the oviposition conditions found in their local environments. Behavioral choice also seems to play a role because females adjusted their oviposition depth in response to soil compaction (both populations) or moisture (eastern population). Eastern females are more likely to encounter saturated soils over large areas and seem to show greater behavioral responses to such conditions than western females. Interestingly, even though females from a larger-bodied population oviposited deeper in loosely compacted soils than females from a smaller-bodied population when adjusting for size, we found no evidence that females from a population with a greater mean body size have greater size-adjusted oviposition depths in hard compacted soils. These patterns suggest that compaction could act as a constraint on oviposition depth, placing a limit on the oviposition depth choices possible for females.

Our results suggest that the population origin of females has a stronger effect on depth than body size for two reasons. First, the positive relationship between oviposition depth and body size was evident in the two field experiments, but not in the laboratory experiment; whereas the population effect on oviposition depth was evident in all three experiments. One explanation for this discrepancy is the plastic response of adult size to the laboratory rearing conditions. Individual *R. microptera*, like many insects, typically

experience their largest increase in size during in the final (fifth) instar; therefore, it is not surprising that we observed a plastic response to the laboratory conditions (S.B. and J.E.J., unpublished data). The females in the laboratory experiment spanned a similar range of adult sizes as observed in the field experiments (pronotum length range: laboratory = 13–23 mm, field = 14–24 mm). The plastic response of body size to laboratory rearing conditions resulted in females from all three populations spanning the entire size range. Despite this size range overlap, we still observed significant differences in oviposition depth among populations, suggesting population origin has a stronger effect on oviposition depth than body size. Second, the size-depth relationship observed in the field is overshadowed by the differences among populations. In the field, females from the two populations overlapped in size, but the extent of the overlap was much smaller than in the laboratory experiment. The slope of the depth-size relationship was similar for both field populations, despite major differences in mean body size (Fig. 4), suggesting a simple, consistent scaling of oviposition depth with size across both populations.

The lack of information pertaining to the adaptive significance of oviposition depth indicates an opportunity for fruitful research. We present here a framework for developing hypotheses and predictions pertaining to adaptive oviposition depth in soil-ovipositing species. Oviposition depth, in part, defines the environmental conditions experienced by eggs, embryos, and hatchlings. For animals that lay eggs in the soil, depth of oviposition is one mechanism by which females can contribute to offspring survival. Oviposition depth should reflect females optimizing at least three selective forces acting on oviposition site choice. First, selection should favor females that minimize the cost of oviposition in time and energy. Time committed to oviposition might result in lower realized fecundity (Janz 2003), prolonged exposure to predation (Michiels and Dhondt 1990), and exposure to detrimental abiotic conditions (Fink and Völk 1995). The energetic demands on females are likely to increase with increasing oviposition depth (Masaki 1986) particularly when soil texture, moisture, and compaction make digging difficult (Doane 1967, Stauffer and Whitman 1997). The time and energetic costs of digging are one explanation for why oviposition depth decreased as compaction increased in our study. All else being equal, minimizing time and energy invested in digging should limit oviposition to relatively shallow depths. Second, selection should favor oviposition depths that improve egg survival in a given environment. Greater protection from surface fires, desiccation, surface-dwelling predators and pathogens, should favor increased oviposition depth. Differences between our sites in soil compaction and moisture (Fig. 1) could affect egg, embryo, and hatchling survival and might account for the observed population differences in oviposition depth. Finally, selection should favor oviposition depths that maximize the probability a hatchling will successfully reach the

surface (Bradford et al. 1993). Two factors would shape the costs of depth to hatchling survival: hatchling size and soil conditions. In *R. microptera*, the egg and hatchling sizes do not differ among these populations (Jannot et al. 2009). Although hatchling size does not vary across the landscape, soil conditions do. Therefore, we predict that the compacted, clay-dominated marl soils at our western sites should inhibit the upward digging of hatchlings (Stauffer and Whitman 1997), whereas the loose, organic peat soils found at our eastern sites should result in easier tunneling for emerging hatchlings. If so, western hatchlings would face more difficult upward digging conditions compared with eastern hatchlings and should favor shallow oviposition depths in western soils due to the energetic costs of digging out of compacted soil and a presumed increased potential for failure to excavate to the surface at greater depths. Quantifying the fitness effects of variation in oviposition depth requires testing the outcome of soil conditions and oviposition depths on both offspring survival and female future reproductive success. We do not currently have such data, but they would be vital for evaluating consequences of oviposition depth choice.

In conclusion, our data are consistent with the hypothesis of local adaptation of female oviposition choice to local environmental conditions. Factors contributing to female ability to choose oviposition depths include both individual size, and behavioral or mechanical traits that vary among populations and are independent of size. For species with limited dispersal and little or no posthatching maternal care, choosing an appropriate depth for oviposition in the vertical soil profile is an important mechanism by which females can influence egg and hatchling survival. Data are lacking for the time and energy necessary for oviposition to any particular depth, the relationship between egg or hatchling survival, oviposition depth and soil conditions. Future work should be directed at a mechanistic understanding of the costs and benefits associated with oviposition depth in soil-ovipositing organisms.

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