Biological Journal of the Linnean Society, 2009, **97**, 494–502. With 2 figures

Local adaptation in a plant herbivore interaction depends on the spatial scale

RODRIGO COGNI* and DOUGLAS J. FUTUYMA

Department of Ecology and Evolution, Stony Brook University, 650 Life Sciences Building, Stony Brook, NY 11794-5245, USA

Received 9 October 2008; accepted for publication 17 December 2008

Local adaptation has central importance in the understanding of co-evolution, maintenance of sexual reproduction, and speciation. We investigated local adaptation in the alkaloid-bearing legume *Crotalaria pallida* and its seed predator, the arctiid moth *Utetheisa ornatrix*, at different spatial scales. When we studied three populations from south-east Brazil (150 km apart), we did not find evidence of local adaptation, although we did find interpopulational differences in herbivore performance, and a significant interaction between herbivore sex and plant population. These results indicate that both moth and plant populations are differentiated at the regional scale. In a comparison of populations from Brazil and Florida, the herbivore showed local adaptation to its host plant; for both moth populations, the pupae were heavier when the larvae ate the sympatric than the allopatric host population. We discuss the scale dependence of our results and the possible causes for the lack of local adaptation at the regional scale, even in the presence of plant and moth differentiation. The results obtained demonstrate the importance of studying co-evolution and local adaptation at different geographical scales. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 494–502.

ADDITIONAL KEYWORDS: co-evolution – gene flow – genetic variation – herbivory – natural selection – Red Queen – seed predation – speciation.

INTRODUCTION

Co-evolution, the reciprocal evolutionary change in interacting species driven by natural selection, is a central theme in studies of interactions among species (Thompson, 2005). Adaptations that have evolved by the interactions among species have enhanced the diversity of life and have affected the structure of ecological communities (Futuyma & Slatkin, 1983). Studies of co-evolutionary dynamics can benefit from spatial variation in the interactions (Thompson, 2005). In antagonistic interactions, the reciprocal selection imposed by co-evolving species leads to a constant shift in the adaptive peaks (Peters & Lively, 2007), and this can result in local adaptation (Kawecki & Ebert, 2004). In parasite–host interactions (i.e. herbivorous insects eating plants), the parasites are expected to exhibit local adaptation owing to their larger population sizes, shorter generation times, and higher mutation rates (Ebert, 1994; Lively, 1999; Dybdahl & Storfer, 2003). Local adaptation is revealed by a higher mean fitness of parasites on local versus foreign hosts or by a higher mean fitness of local parasites than foreign parasites on local hosts (Williams, 1966; Kawecki & Ebert, 2004). This dynamic nature of local adaptation among co-evolving species is an important mechanism in many theories within evolutionary biology, including explanations for the maintenance of genetic variation, the maintenance of sexual reproduction, and the processes of parapatric and sympatric speciation (Kawecki & Ebert, 2004).

Empirical studies of local adaptation in host–parasite interactions show highly variable outcomes. Although many studies have detected parasite local adaptation, others failed to detect local adaptation, or even found parasites to be locally maladapted (Greischar & Koskella, 2007; Hoeksema & Forde, 2008).

---

*Corresponding author. E-mail: rcogni@life.bio.sunysb.edu

This variation is expected because local adaptation can be influenced by several factors. First, migration rates of both the host and the parasite can influence local adaptation. Theoretical models show that, if the migration rate of parasite is higher than that of the host, the parasite will be locally adapted; however, if the host has higher migration, the host will be locally adapted (Gandon, 2002). Indeed, this prediction was confirmed by two recent meta-analyses of the available empirical studies (Greischar & Koskella, 2007; Hoeksema & Forde, 2008). This appears to be counterintuitive because gene flow can swamp local adaptation. However, factors that increase the evolutionary potential of the parasite increase its ability to track local host allele frequencies (Dybdahl & Storfer, 2003).

Local adaptation can also vary across spatial scales. Many negative results on local adaptation studies may be the consequence of co-evolutionary process occurring at either larger or smaller spatial scales (Thrall, Burdon & Bever, 2002). Assuming that physical distance corresponds with genetic differences among populations, the distance among populations is predicted to be negatively correlated with adaptation (Gandon *et al.*, 1996). Therefore, parasites are predicted to be more adapted to hosts from nearby populations than to those that are further away (Kaltz & Shykoff, 2002). A few empirical studies confirm the prediction that local adaptation is more likely to be detected at a larger than a smaller scale (Hanks & Denno, 1994; Burdon & Thompson, 1995; Thrall *et al.*, 2002; Stenberg, Witzell & Ericson, 2006; Stenberg & Axelsson, 2008). However, very few studies of local adaptation have investigated populations at different scales. Most studies of local adaptation only include populations less than 1000 km apart (Hoeksema & Forde, 2008), and there has been no investigation on local adaptation at very large scales, such as continental comparisons with populations several thousand kilometers apart.

In the present study, we investigated local adaptation in the interaction between the alkaloid-bearing legume *Crotalaria pallida* (Fabaceae: Papilionoideae) and its seed predator, the arctiid moth *Utetheisa ornatrix* (Lepidoptera: Arctiidae). We conducted a common-garden experiment with reciprocal combinations of host and herbivore populations at both regional and continental scales, and demonstrate that local adaptation depends on the geographical scale.

MATERIAL AND METHODS

STUDY SYSTEM

Crotalaria is a pantropical genus of weeds with approximately 600 species (Polhill, 1982). *Crotalaria pallida* is a species native from Africa, which now occurs at high densities from southern Brazil to the south-eastern USA. There is no clear evidence about the New World introduction; possibly, it was transported from Africa during the slave trade in the 16th Century (Polhill, 1982). *Crotalaria pallida* constitutively produces pyrrolizidine alkaloids (PAs) (Wink & Mohamed, 2003). PAs are assumed to serve as protective chemicals against herbivores (Hartmann, 2004), and the toxic effect of PAs on a generalist herbivore has recently been demonstrated (Narberhaus, Zintgraf & Dobler, 2005). *Crotalaria pallida* also has extrafloral nectaries that attract ants, which positively affect plant fitness by decreasing herbivore activity (Heil & McKey, 2003; Ferro, Guimarães & Trigo, 2006; Guimarães *et al.*, 2006).

Utetheisa ornatrix is a neotropical species that specializes on the genus *Crotalaria*. It originally fed on native *Crotalaria* species, but its main host is now *C. pallida* in several locations (Ferro, 2001; Eisner, 2003). In all the areas included in the present study, *U. ornatrix* occasionally uses the other *Crotalaria* species that occur at low densities. *Utetheisa ornatrix* sequester PAs from the host plant. The PAs not only protect larvae and adults, but also are transmitted from the female (and from males through nuptial gift) to eggs (Eisner, 2003). Males also modify the PAs into a courtship pheromone (Dussourd *et al.*, 1991; Iyengar & Eisner, 1999a, b). *Utetheisa ornatrix* can have a significant impact on the fitness of *Crotalaria* plants. Even though the larvae are mobile and can walk about the plant and feed on leaves, most of the larvae are found inside the fruit preying on the seeds (Ferro *et al.*, 2006). Up to 20% of *C. pallida* fruits in the field may be damaged by *U. ornatrix* (Ferro, 2001).

POPULATIONS STUDIED AND ORGANISM MAINTENANCE

In May 2005, we collected *C. pallida* seeds and moths from three populations in São Paulo State, Southeast Brazil: Campinas (22°47’S, 47°04′W; 680 m a.s.l.); Juquiá (24°19’S, 47°38′W; 25 m a.s.l.); and the district of Vitoriana in Botucatu (22°47’S, 48°24′W, 578 m a.s.l.). These populations are referred to as CA, JU, and BO, respectively. JU is 200 km from BO, and 186 km from CA. CA is 160 km from BO. In April 2006, we collected from another population at Archbold Biological Station (27°15′N, 81°21’S; 30 m a.s.l.), in central Florida, USA. For each population, we collected seeds from at least 30 individuals and a minimum of 40 adult moths.

We maintained a large colony of moths from each population (with more than 25 adults at any single time) in the laboratory. We fed non-experimental larvae on an artificial diet based on *Phaseolus* beans

(Signoretti *et al.*, 2008) to avoid maternal and paternal effects, and to avoid selection for the use of the host plant population available in captivity. We kept adults in paper cages (approximately 3.2 litres), where 5% honey solution was provided.

We carried out two sets of experiments: in 2005, we studied the three populations from Brazil and, in 2006, we studied one population from Brazil (CA) and the population from Florida. We grew plants from seeds in a common environment. Seeds were germinated in an incubator at 26 °C and 24 h of light. Seedlings were transferred to trays (approximate volume 650 mL) filled with standard potting soil. After 3 weeks, we transferred seedlings to large pots (approximately 7.6 litres). Seedlings and plants were kept in a greenhouse under natural sunlight. Plants were watered daily and standard fertilizer (15N : 5P : 15K at 300 p.p.m.) was added weekly. In the 2005 experiment, we used the greenhouse of the Instituto de Biologia at Universidade Estadual de Campinas (São Paulo State, Brazil) without a temperature control. In the 2006 experiment, we used the greenhouse at the Life Science Building at Stony Brook University (Stony Brook, NY) with an approximate temperature of 28 °C (day) and 24 °C (night). Even if it is very likely that the conditions of the greenhouses in the two different countries differed, such differences did not affect our results. When we compared the same treatment carried out in 2005 and 2006 (moths from CA eating plants from CA), there was no significant effect of the greenhouse (2005 in Brazil versus 2006 in Stony Brook) on any of the response variables (pupal weight: $F = 2.77$, d.f. = 1, $P = 0.10$; development time: $F = 2.25$, d.f. = 1, $P = 0.14$; total number of seeds consumed: $F = 2.98$, d.f. = 1, $P = 0.09$; weight of seeds consumed: $F = 3.4$, d.f. = 1, $P = 0.07$).

Table 1. Percent survival of *Utetheisa ornatrix* larvae eating *Crotalaria pallida* in a laboratory experiment

		Plants from		
		BO	CA	JU
Moths from	BO	0.24 (128)	0.24 (133)	0.20 (133)
	CA	0.21 (131)	0.29 (139)	0.30 (129)
	JU*	0.12 (108)	0.16 (134)	0.16 (140)

*Moths from JU showed a significant lower survival on a nominal logistic model.

Sample sizes (number of neonate larvae) are giving in parenthesis. Moth and plant populations are from three localities in São Paulo State, south-east Brazil.

BO, Botucatu; CA, Campinas; JU, Juquiá.

Table 2. Percent survival of *Utetheisa ornatrix* larvae eating *Crotalaria pallida* in a laboratory experiment

		Plants from	
		CA	FL
Moths from	CA	0.33 (160)	0.34 (179)
	FL*	0.18 (164)	0.18 (162)

*Moths from Florida showed a significant lower survival on a nominal logistic model.

Sample sizes (number of neonate larvae) are giving in parenthesis. Moth and plant populations are from Campinas (CA) in São Paulo State in south-east Brazil and Central Florida (FL) in the USA.

EXPERIMENT DETAILS

In 2005, we started the plants in May and carried out the experiments from September to December. In 2006, we started the plants in April and carried out the experiments from August to November. We carried out all experiments on an incubator at 26 °C. We fed larvae from each population from hatching to pupation on each host population. In 2005, we studied three populations from Brazil (BO, CA, and JU), such that there were nine treatments (each of the three moth populations feeding on each of the three plant populations). In 2006, we studied one population from Brazil (CA) and one population from Florida, such that there were four treatments. Sample sizes for each treatment are given in Tables 1, 2. We fed larvae on fresh leaves for the first 4 days and, subsequently, on green fresh fruits. This simulates conditions in the wild, where neonates consume leaf material before getting inside the fruit (Ferro, 2001; Eisner, 2003). We placed neonate larvae individually in a 1.5-mL microcentrifuge tube with a leaf-disc. We provided a new leaf-disc after 48 h. Leaf-discs were made from fresh leaves and were 1 cm in diameter. After 4 days of eating leaves, larvae were transferred to individual Petri dishes (5 cm diameter) with a moistened filter paper and green fruit. We opened fruits with a razor blade to completely expose the seeds. Every other day, we transferred the larva to a clean dish and provided new fruit. The amount of fruit given to each larva was: one-third of a fruit on days 5 and 7, one-half of a fruit on day 9, 1 on days 11 and 13, and two fruits (every other day) after day 13. We weighed each fruit (to the nearest 0.001 g) before and after each 48-h period. Fruits lost approximately 20% of weight as a result of water loss during the 48 h; however, water loss did not differ among the host populations. We also counted the number of seeds on each fruit provided to each larva and the number of seeds not eaten during each 2-day period to calculate the total number of seeds consumed per larva. We recorded larval development time as the number of days that each larva took from egg hatching to pupation. We recorded larval survival as the percentage of neonate larvae that survived to pupation. We measured pupal weight 5 days after pupation. Pupal weight correlates with adult weight and adult fitness in *U. ornatrix* (Iyengar & Eisner, 1999b); larger females lay more eggs and large males attract more females to mate.

STATISTICAL ANALYSIS

We tested the effect of moth population, plant population, and the interaction of plant and moth population on larval survival with a nominal logistic model (Hosmer & Lemeshow, 1989). We tested the effect of plant population, moth population, sex and all possible interactions on each response variable (pupal weight, larval development time, total number of seeds consumed per larva, and total weight of seeds consumed per larva) by analysis of variance. We define local adaptation as a significant interaction between the herbivore source population and the host source population, in which, on average, individual herbivores exhibit higher fitness on its native host population than on hosts from allopatric populations.

RESULTS

THREE POPULATIONS FROM BRAZIL (2005)

No evidence of local adaptation was detected in this experiment (i.e. no significant interaction between plant population and moth population). Moths from JU showed a lower survival than the moths from the other two populations, and survival was not affected by plant population or the interaction between plant population and moth population (effect likelihood ratio tests in a nominal logistic model: moth population $\chi^2 = 21.54$, d.f. = 2, $P < 0.0001$; plant population $\chi^2 = 1.32$, d.f. = 2, $P = 0.51$; plant population × moth population $\chi^2 = 3.23$, d.f. = 4, $P = 0.52$; Table 1). Moths from JU also had lower pupal weight and longer development time than the other two moth populations (Table 3). The weight of seeds consumed was slightly higher for larvae from CA (Table 3). Pupal weight was higher for males than for females (Table 3). Interactions between plant population and moth sex on herbivore performance indicate that plant quality varies among populations. Pupal weight, development time, and the number of seeds consumed were significantly affected by the interaction of plant population and sex (Fig. 1, Table 3) (i.e. the sexes showed different responses to differences in plant population). Although males showed higher

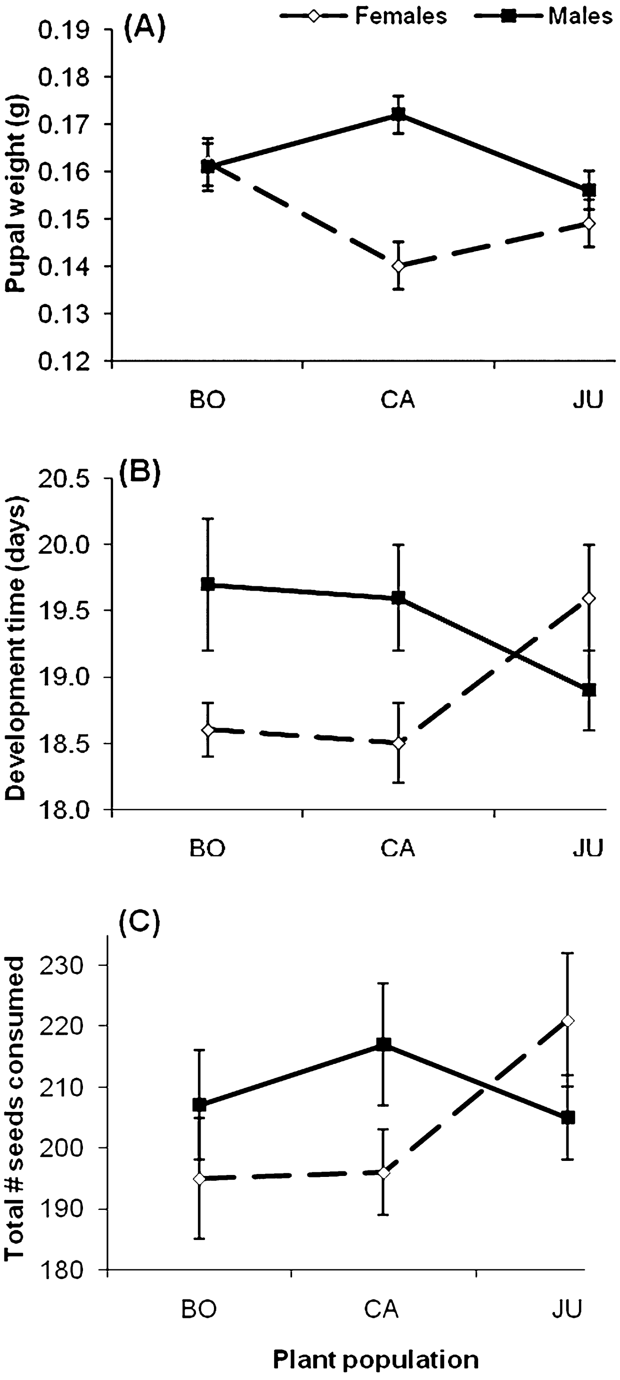

Figure 1. Interaction between sex and plant population on (A) pupal weight, (B) development time, and (C) total number of seeds consumed for larvae of *Utetheisa ornatrix* feeding on *Crotalaria pallida* fruits in a laboratory experiment. Moth and plant populations are from three localities in São Paulo State, south-east Brazil. BO, Botucatu; CA, Campinas; JU, Juquiá. Data are the mean ± SE.

Table 3. Effect of plant source population, moth source population, sex, and all possible interactions on pupal weight, larval development time, total number of seeds consumed, and weight of seeds consumed

	Three populations from Brazil (2005)			Brazil versus Florida (2006)		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*
Pupal weight						
Plant population	2	0.88	0.415	1	0.14	0.709
Moth population	2	14.82	0.000*	1	0.29	0.589
Sex	1	18.26	0.000*	1	9.26	0.003*
Plant population × Moth population	4	0.10	0.984	1	11.51	0.001*
Plant population × Sex	2	6.47	0.002*	1	0.70	0.402
Moth population × Sex	2	0.45	0.634	1	0.20	0.651
Plant population × Moth Population × Sex	4	2.96	0.020*	1	0.34	0.561
Error	252			163		
Development time						
Plant population	2	0.21	0.808	1	0.01	0.938
Moth population	2	3.12	0.046*	1	1.43	0.233
Sex	1	2.73	0.100	1	0.40	0.525
Plant population × Moth population	4	1.13	0.341	1	2.32	0.130
Plant population × Sex	2	4.41	0.013*	1	1.39	0.241
Moth population × Sex	2	0.15	0.862	1	0.46	0.501
Plant population × Moth Population × Sex	4	1.51	0.201	1	2.44	0.120
Error	252			163		
Number of seeds consumed						
Plant population	2	1.57	0.211	1	43.67	0.000*
Moth population	2	1.71	0.184	1	0.001	0.980
Sex	1	1.20	0.274	1	1.11	0.294
Plant population × Moth population	4	1.54	0.190	1	0.004	0.949
Plant population × Sex	2	3.54	0.031*	1	0.25	0.873
Moth population × Sex	2	0.33	0.718	1	0.22	0.636
Plant population × Moth Population × Sex	4	1.87	0.116	1	0.57	0.450
Error	252			163		
Weight of seeds consumed						
Plant population	2	0.02	0.984	1	0.65	0.422
Moth population	2	4.34	0.014*	1	0.29	0.593
Sex	1	0.75	0.389	1	0.13	0.719
Plant population × Moth population	4	1.22	0.303	1	0.089	0.766
Plant population × Sex	2	2.53	0.082	1	0.142	0.706
Moth population × Sex	2	1.05	0.351	1	0.052	0.821
Plant population × Moth Population × Sex	4	0.48	0.747	1	0.821	0.366
Error	252			163		

Utetheisa ornatrix larvae were fed with fruits of *Crotalaria pallida* in the laboratory. Three populations from Brazil: moth and plant populations are from three localities in Sao Paulo State, south-east Brazil. Brazil versus Florida: moth and plant populations are from Campinas in Sao Paulo State in south-east Brazil and central Florida in the USA.
d.f., degrees of freedom.
*, $P < 0.05$.

pupal weight on plants from CA, females had the lowest pupal weight in this plant population (Fig. 1A). Females took longer to develop and ate more seeds when feeding on the JU plants, whereas males had the lowest development time and lowest seed consumption in this plant population (Fig. 1B, C).

Brazil versus Florida (2006)

Local adaptation of *U. ornatrix* to its host plant was detected in the experiment with populations from Brazil and Florida; pupae from each population were heavier when reared on the sympatric than the allo-

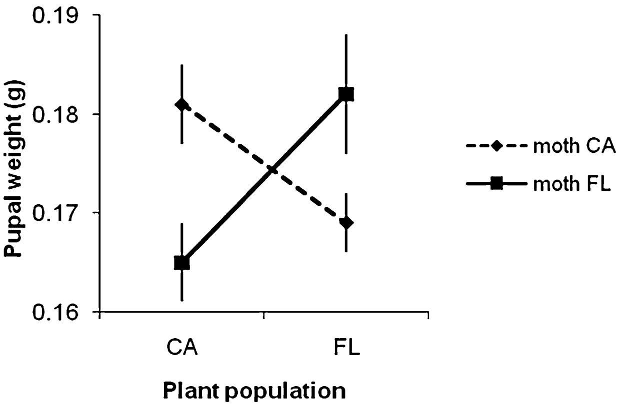

Figure 2. Pupal weight of *Utetheisa ornatrix* feeding on *Crotalaria pallida* fruits in a laboratory experiment. Moth and plant populations are from Campinas (CA) in São Paulo State in south-east Brazil and central Florida (FL) in the USA. Data are the mean ± SE.

patric host population (Fig. 2, Table 3). Pupal weight was higher for males than for females (Table 3). Moths from Florida showed a lower survival than moths from Brazil, and survival was not affected by plant population or the interaction between plant population and moth population (effect likelihood ratio tests in a nominal logistic model: moth population $\chi^2 = 17.34$, d.f. = 1, $P < 0.0001$; plant population $\chi^2 = 0.24$, d.f. = 1, $P = 0.62$; plant population × moth population $\chi^2 = 0.0002$, d.f. = 1, $P = 0.99$; Table 2). Development time and the weight of seeds consumed did not significantly vary among the treatments (Table 3). A higher number of seeds was consumed for the Brazilian plants than for the plants from Florida (Table 3).

DISCUSSION

In the present study, we did not find local adaptation at a regional scale, but we could detect both moth and plant population differences at this scale. First, one moth population showed a consistently lower performance than the other two. Second, we found significant interactions between herbivore sex and plant population on several performance traits of the herbivore. These interactions indicate that the plant populations are not uniform at this scale. Some previous studies have reported differential responses of male and females to host quality (Tikkanen, Niemela & Keranen, 2000; Jormalainen *et al.*, 2001; Asshoff & Hättenschwiler, 2005), and these differences were attributed to sexual selection and differences in optimal reproductive strategies. Assuming sequestration is costly at high levels (Bowers, 1992), *U. ornatrix* males and females are likely under stabilizing selection to acquire enough PAs for protection, but males may also be under runaway sexual selection to sequester higher amounts of PAs for courtship (Iyengar & Eisner, 1999a). Future studies should explore this hypothesis by investigating differences in PA content among plant populations, and any possible differences in the ability of the sexes to sequester PAs.

Even though we found both moth and plant population differences at a regional scale, we were unable to detect local adaptation at this level. In other plant herbivore systems, empirical studies have found herbivore local adaptation, herbivore local maladaptation, or neither evidence for local adaptation or maladaptation (Karban, 1989; Zangerl & Berenbaum, 1990; Strauss, 1997; Roy, 1998). In our system, why is local adaptation expected even at a regional scale and why did we not detect it? First, we used the best design for the detection of local adaptation: a common-garden with reciprocal design and large sample sizes (Thrall *et al.*, 2002; Blanford *et al.*, 2003; Kawecki & Ebert, 2004; Laine, 2007). We chose a common-garden design because it tests spatial covariance between the genotype frequencies of the interacting species. This reflects how parasites track their host over evolutionary time (i.e. Red Queen co-evolution) (Kawecki & Ebert, 2004; Nuismer & Gandon, 2008). However, common-garden designs do not test for local adaption of the host and the parasites to their sympatric ecological environment, such as abiotic conditions and interactions with other species (Ridenhour & Nuismer, 2007; Nuismer & Gandon, 2008). This may be important when adaptations to the host plant quality are also mediated by adaptation to other ecological variables (Laine, 2008).

Second, local adaptation depends on the strength of selection as a result of the interaction (Nuismer, Thompson & Gomulkiewicz, 2000); for example, the best example of local adaptation in a plant–insect interaction is the parsnip webworm that imposes strong selection on wild parsnip by feeding exclusively on the host's reproductive structures (Berenbaum, Zangerl & Nitao, 1986; Berenbaum & Zangerl, 1998). In our system, the reciprocal selection as a result of the interaction is also believed to be strong. The moth depends on the plant for food and PAs (Eisner, 2003), and *U. ornatrix* feeds mostly on the fruits, greatly decreasing the plant seed set (Ferro, 2001).

Third, the level of specificity on the interaction can also influence local adaptation (Gandon, 2002). In the interaction between parsnips and the parsnip webworm, there are relatively few other herbivores and a rare alternative host plant (Zangerl & Berenbaum, 2003). In our system, there is also a high level of specificity. *U. ornatrix* is the main herbivore on *C. pallida* plants and *C. pallida* is the main host for

U. ornatrix throughout the neotropics (Ferro, 2001; Eisner, 2003).

Fourth, demography can influence the patterns of local adaptation. High levels of migration can prevent local adaptation (Gandon *et al.*, 1996), as occurs in the pinyon needle scale (Cobe & Whitham, 1998). In our system, both species are spatially differentiated at the regional scale. Theoretical models also predict that, in the arms-race between the host and the parasite, the one with higher migration rate is expected to be locally adapted (Gandon, 2002). *Crotalaria pallida* is an annual plant with limited dispersal; this species is partially selfing and bee pollinated, and lacks any mechanism for long-distance seed dispersal. Short-distance differentiation can evolve in a few generations for plants with such traits (Heywood, 1991; Linhart & Grant, 1996). On the other hand, flying insect herbivores have higher dispersal abilities; therefore, we expect local adaptation by the herbivore in our system. We are currently investigating the genetic variation and population structure of *C. pallida* and *U. ornatrix* using molecular markers. Additionally, future studies might address whether local adaptation is influenced by demographic stochasticity, such as genetic drift, founder effects and meta-population extinction, as well as by temporal variability in natural selection, and whether it is constrained by the lack of genetic variation and the genetic architecture of traits relevant to the interaction (Kawecki & Ebert, 2004).

The present study shows that local adaptation depends on the geographical scale; although we did not find local adaptation at a regional scale, we found evidence of local adaptation at a continental scale. We expect the host plant to be more differentiated at a continental scale. For example, several salt marsh plants have evolved latitudinal differences in palatability to herbivores along North America East coast (Salgado & Pennings, 2005). Another possible explanation for the scale dependence of our results might be that, when individual parasite populations are ephemeral, local adaptation may only be found at larger geographical scales (Thrall & Burdon, 1997; Burdon & Thrall, 2000). Indeed, *C. pallida* has a patchy distribution and occurs in habitats where fire and other human disturbances that cause local moth extinction are common.

A few other studies report similar results in which local adaptation was detected only at larger scales (Ebert, 1994; Hanks & Denno, 1994; Burdon & Thompson, 1995; Thrall *et al.*, 2002; Stenberg *et al.*, 2006; Stenberg & Axelsson, 2008; for a counter example, see also Imhoof & Schmid-Hempel, 1998). However, Lively (1989) found local adaptation even between nearby populations and Laine (2005) found local adaptation to sympatric host populations and also found that local adaptation may extend to the scale of the sympatric host metapopulation. Our results also demonstrate the importance of studying co-evolution and local adaptation at different geographical scales; otherwise, co-evolutionary dynamics occurring at either larger or smaller scales might be missed.

ACKNOWLEDGEMENTS

We thank J. R. Trigo for providing advice on the system, laboratory space, and help with permits; C. Haddad and Instituto de Biologia (Unicamp) for providing greenhouse space; M. A. Son for help in the laboratory; and A. R. Moraes, M. F. Pereira, W.W. Benson (Unicamp), and M. Deyrup (Archbold Biological Station) for help with the collection and transportation of moths. This manuscript was improved by comments from A. L. Laine and anonymous referees. We also thank IBAMA and USDA for permits to export and import live organisms. We thank FERTL (Functional Ecology Research and Training Laboratory) for use of equipment. Financial support was provided by the Latin American and Caribbean Studies at SUNY Stony Brook and the Hovanitz Memorial Award Program from the Lepidoptera Foundation. This work is contribution number 1178 in Ecology and Evolution from Stony Brook University.

REFERENCES

Asshoff R, Hättenschwiler S. 2005. Growth and reproduction of the alpine grasshopper *Miramella alpina* feeding on Co_2-enriched dwarf shrubs at treeline. *Oecologia* **142:** 191–201.

Berenbaum MR, Zangerl AR. 1998. Chemical phenotype matching between a plant and its insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America* **95:** 13743–13748.

Berenbaum MR, Zangerl AR, Nitao JK. 1986. Constraints on chemical co-evolution – wild parsnips and the parsnip webworm. *Evolution* **40:** 1215–1228.

Blanford S, Thomas MB, Pugh C, Pell JK. 2003. Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. *Ecology Letters* **6:** 2–5.

Bowers MD. 1992. The evolution of unpalatability and the cost of chemical defense in insects. In: Roitberg BD, Isman MB, eds. *Insect chemical ecology, an evolutionary approach*. New York, NY: Chapman and Hall, 216–244.

Burdon JJ, Thompson JN. 1995. Changed patterns of resistance in a population of *Linum marginale* attacked by the rust pathogen *Melampsora lini*. *Journal of Ecology* **83:** 199–206.

Burdon JJ, Thrall PH. 2000. Co-evolution at multiple spatial scales: *Linum marginale–Melampsora lini* – from the individual to the species. *Evolutionary Ecology* **14:** 261–281.

Cobe NS, Whitham TG. 1998. Prevention of deme formation by the pinyon needle scale: problems of specializing in a dynamic system. In: Mopper S, Strauss SY, eds. *Genetic structure and local adaptation in natural insect populations*. New York, NY: Chapman and Hall, 37–63.

Dussourd DE, Harvis CA, Meinwald J, Eisner T. 1991. Pheromonal advertisement of a nuptial gift by a male moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences of the United States of America* **88:** 9224–9227.

Dybdahl MF, Storfer A. 2003. Parasite local adaptation: Red Queen versus Suicide King. *Trends in Ecology & Evolution*, **18:** 523–530.

Ebert D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science* **265:** 1084–1086.

Eisner T. 2003. *For love of insects*. Cambridge, MA: Harvard University Press.

Ferro VG. 2001. *Padrões de utilização de* Crotalaria *spp. (Leguminosae, Papilionoideae, Crotalarieae) por larvas de* Utetheisa ornatrix *(Lepidoptera, Arctiidae)*. Masters Thesis, Universidade Estadual de Campinas.

Ferro VG, Guimarães PR, Trigo JR. 2006. Why do larvae of *Utetheisa ornatrix* penetrate and feed in pods of *Crotalaria* species? Larval performance vs. chemical and physical constraints. *Entomologia Experimentalis Et Applicata* **121:** 23–29.

Futuyma DJ, Slatkin M. 1983. *Co-evolution*. Sunderland, MA: Sinauer Associates.

Gandon S. 2002. Local adaptation and the geometry of host-parasite co-evolution. *Ecology Letters* **5:** 246–256.

Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I. 1996. Local adaptation and gene-for-gene co-evolution in a metapopulation model. *Proceedings of the Royal Society of London Series B, Biological Sciences* **263:** 1003–1009.

Greischar MA, Koskella B. 2007. A synthesis of experimental work on parasite local adaptation. *Ecology Letters* **10:** 418–434.

Guimarães PR, Raimundo RLG, Bottcher C, Silva RR, Trigo JR. 2006. Extrafloral nectaries as a deterrent mechanism against seed predators in the chemically protected weed *Crotalaria pallida* (Leguminosae). *Austral Ecology* **31:** 776–782.

Hanks LM, Denno RF. 1994. Local adaptation in the armored scale insect *Pseudaulacaspis pentagona* (Homoptera, Diaspididae). *Ecology* **75:** 2301–2310.

Hartmann T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta* **219:** 1–4.

Heil M, McKey D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology Evolution and Systematics* **34:** 425–453.

Heywood JS. 1991. Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* **22:** 335–355.

Hoeksema JD, Forde SE. 2008. A meta-analysis of factors affecting local adaptation between interacting species. *The American Naturalist* **171:** 275–290.

Hosmer DW, Lemeshow S. 1989. *Applied logistic regression*. New York, NY: John Wiley & Sons.

Imhoof B, Schmid-Hempel P. 1998. Patterns of local adaptation of a protozoan parasite to its bumblebee host. *Oikos* **82:** 59–65.

Iyengar VK, Eisner T. 1999a. Female choice increases offspring fitness in an arctiid moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences of the United States of America* **96:** 15013–15016.

Iyengar VK, Eisner T. 1999b. Heritability of body mass, a sexually selected trait, in an arctiid moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences of the United States of America* **96:** 9169–9171.

Jormalainen V, Honkanen T, Makinen A, Hemmi A, Vesakoski O. 2001. Why does herbivore sex matter? Sexual differences in utilization of *Fucus vesiculosus* by the isopod *Idotea baltica*. *Oikos* **93:** 77–86.

Kaltz O, Shykoff JA. 2002. Within- and among-population variation in infectivity, latency and spore production in a host-pathogen system. *Journal of Evolutionary Biology* **15:** 850–860.

Karban R. 1989. Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature* **340:** 60–61.

Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7:** 1225–1241.

Laine AL. 2005. Spatial scale of local adaptation in a plant-pathogen metapopulation. *Journal of Evolutionary Biology* **18:** 930–938.

Laine AL. 2007. Detecting local adaptation in a natural plant-pathogen metapopulation: a laboratory vs. field transplant approach. *Journal of Evolutionary Biology* **20:** 1665–1673.

Laine AL. 2008. Temperature-mediated patterns of local adaptation in a natural plant-pathogen metapopulation. *Ecology Letters* **11:** 327–337.

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27:** 237–277.

Lively CM. 1989. Adaptation by a parasitic trematode to local populations of its snail host. *Evolution* **43:** 1663–1671.

Lively CM. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. *American Naturalist* **153:** S34–S47.

Narberhaus I, Zintgraf V, Dobler S. 2005. Pyrrolizidine alkaloids on three trophic levels – evidence for toxic and deterrent effects on phytophages and predators. *Chemoecology* **15:** 121–125.

Nuismer SL, Gandon S. 2008. Moving beyond common-garden and transplant designs: insights into causes of local adaptation in species interactions. *American Naturalist* **171:** 658–668.

Nuismer SL, Thompson JN, Gomulkiewicz R. 2000. Co-evolutionary clines across selection mosaics. *Evolution* **54:** 1102–1115.

Peters AD, Lively CM. 2007. Short and long term benefits and detriments to recombination under antagonistic co-evolution. *Journal of Evolutionary Biology* **20:** 1206–1217.

Polhill RM. 1982. *Crotalaria in Africa and Madagascar*. Kew: Royal Botanical Gardens.

Ridenhour BJ, Nuismer SL. 2007. Polygenic traits and parasite local adaptation. *Evolution* **61:** 368–376.

Roy BA. 1998. Differentiating the effects of origin and frequency in reciprocal transplant experiments used to test negative frequency-dependent selection hypotheses. *Oecologia* **115:** 73–83.

Salgado CS, Pennings SC. 2005. Latitudinal variation in palatability of salt-marsh plants: are differences constitutive? *Ecology* **86:** 1571–1579.

Signoretti AGC, Nava DE, Bento JMS, Parra JRP. 2008. Biology and thermal requirements of *Utetheisa ornatrix* (L.) (Lepidoptera: Arctiidae) reared on artificial diet. *Brazilian Archives of Biology and Technology* **51:** 647–653.

Stenberg JA, Axelsson EP. 2008. Host race formation in the meadowsweet and strawberry feeding leaf beetle *Galerucella tenella*. *Basic and Applied Ecology* **9:** 560–567.

Stenberg JA, Witzell J, Ericson L. 2006. Tall herb herbivory resistance reflects historic exposure to leaf beetles in a boreal archipelago age-gradient. *Oecologia* **148:** 414–425.

Strauss SY. 1997. Lack of evidence for local adaptation to individual plants clones or site by a mobile specialist herbivore. *Oecologia* **110:** 77–85.

Thompson JN. 2005. *The geographic mosaic of Co-evolution*. Chicago, IL: The University of Chicago Press.

Thrall PH, Burdon JJ. 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. *Journal of Ecology* **85:** 743–753.

Thrall PH, Burdon JJ, Bever JD. 2002. Local adaptation in the *Linum marginale-Melampsora lini* host-pathogen interaction. *Evolution* **56:** 1340–1351.

Tikkanen OP, Niemela P, Keranen J. 2000. Growth and development of a generalist insect herbivore, *Operophtera brumata*, on original and alternative host plants. *Oecologia* **122:** 529–536.

Williams GC. 1966. *Adaptation and natural selection*. Princeton, NJ: Princeton University Press.

Wink M, Mohamed GIA. 2003. Evolution of chemical defense traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the rbcL gene. *Biochemical Systematics and Ecology* **31:** 897–917.

Zangerl AR, Berenbaum MR. 1990. Furanocoumarin induction in wild parsnip: genetics and populational variation. *Ecology* **71:** 1933–1940.

Zangerl AR, Berenbaum MR. 2003. Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. *Evolution* **57:** 806–815.