Heredity **83** (1999) 127–137 *Received 20 November 1998, accepted 18 March 1999*

Natural selection and genetic differentiation of behaviour between parasitoids from wild and cultivated habitats

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Differences in behaviour between individuals in populations living in different environments may result from evolution proceeding differently in each population. The parasitoid wasp *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) parasitizes early instar larvae of butterflies in the family Pieridae. In the study area the only host of *C. glomerata* is the Small Cabbage White Butterfly [*Pieris rapae* (L.) (Lepidoptera: Pieridae)], which feeds on cruciferous host plants in a variety of habitats. The behaviour of this parasitoid wasp collected from two habitat types (wild and agricultural) was observed in a reciprocal transplant-style experiment in a greenhouse. Differences in behaviour between wasp sources and test habitat type were analysed using canonical analysis in multivariate analysis of variance. Directional selection on parasitoid behaviour in each test habitat type was estimated by regressing the relative rate of parasitism (a measure of relative fitness) on the behavioural character state. We found that there is genetic differentiation of behaviour between wasps from wild and cultivated habitats and that a different set of behaviours is associated with short-term fitness within models of each source habitat. There was no evidence of local adaptation of wasps to either habitat.

Keywords: *Cotesia glomerata*, evolution, foraging behaviour, *Pieris rapae*, tritrophic interaction.

Introduction

Natural selection in different habitat types can lead to genetic divergence and local adaptation of populations. Thus, if there is sufficient genetic variability of behaviour and a relatively small amount of gene flow, populations in different environments can evolve to behave differently. A parasitoid wasp forages for hosts in which to oviposit, and the ability to parasitize hosts successfully depends, in part, on the wasp's behaviour. Because environments may differ in the behaviours that lead to successful parasitism, wasps in different habitats may evolve to behave differently in response to natural selection.

Although little is known of the details of behaviour of parasitoid wasps at the level of foraging among plants (Godfray, 1994), specific aspects of the environment such as host and host plant odour are known to influence parasitoid behaviour (Turlings *et al*., 1991; Godfray, 1994). In the laboratory, parasitoid wasps have been shown to orientate upwind towards odour sources (see van Alphen & Vet, 1986; Turlings *et al*., 1991; Benrey *et al*., 1997), and to orientate towards forms and colours (Wackers & Lewis, 1994), which may aid them in finding suitable hosts. However, studies outside of the laboratory are limited. Field experiments have shown that attributes of the habitat, such as complexity (Landis & Haas, 1992) or plant species (Pimentel, 1961; see also Godfray, 1994), influence the field parasitism rate. However, in most field studies, one cannot distinguish between the number of wasps in a habitat and the success of individuals in that habitat as causes of a given parasitism rate. Moreover, the specific behaviours that are associated with individual rate of parasitism in different natural habitats are essentially unknown.

Previous studies of genetic differentiation in behaviour between populations of parasitoid wasps have primarily been performed using laboratory colonies (such as Chassain & Bouletreau, 1991; Henter *et al*., 1996). These populations may have experienced selection in the laboratory for many generations, and therefore they may no longer represent the genetic variability found in the wild. Among the handful of

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previous studies of natural populations (Chassain *et al*., 1988; Kester & Barbosa, 1994; Fleury *et al*., 1995) the fitness consequences of divergent behaviour have not been addressed. Additionally, the conditions under which parasitoid behaviour has been studied have generally been so simplified that the observed results cannot be interpreted in the complex ecological context in which evolution takes place (but see Luna & Prokopy, 1995).

Although the study of natural selection is dependent on observable variability of phenotypic traits (Lande & Arnold, 1983), the large expected variability of behaviour within individuals has made the measurement of natural selection on complex behaviour of insects daunting to evolutionary biologists. Nonetheless, because behaviour is such an important component of insect ecology and evolution, it is crucial to attempt an analysis of how it may be affected by natural selection.

Behaviour is most realistically addressed by observing many behavioural components simultaneously because natural selection acts on the entire phenotype, not on isolated components (Brodie & Janzen, 1995). Multiple regression has been used in the study of natural selection on a variety of different types of traits (e.g. Lande & Arnold, 1983; Conner, 1988). Some of these studies have included both behavioural and morphological characters (e.g. Conner, 1988), but we have found none to date that has specifically investigated the possibility that selection on behaviour may differ between environments.

Our experiment was designed to test: (i) whether parasitoids would behave differently when tested in a cabbage habitat than when exposed to a wild host plant habitat; (ii) whether the observed behaviour of parasitoids would depend in part on where they were collected from (henceforth, the 'source' habitat); (iii) whether parasitoid populations were locally adapted, and whether the two habitats act as measurably different selective environments with respect to wasp behaviour; and (iv) we asked whether the same behaviours that we found to be associated with short-term fitness in each test habitat were genetically differentiated among populations.

Towards this end, we observed the behaviour and measured the parasitism rate of individual parasitoid wasps from four populations. Two of these populations were from cabbage fields in an agricultural area dominated by hundred-acre monocultures of cabbage. The other two were from streamside habitats containing wild host plants. The reciprocal transplant style experiment took place in relatively large model habitats in a greenhouse, which were designed to mimic much of the complexity of the natural settings. We thus attempted to address variation in behaviour in an experimental setting close to that in which we believe evolution has occurred.

Materials and methods

Natural history

The Small White Cabbage Butterfly [*Pieris rapae* (L.) (Lepidoptera: Pieridae)] is native to Europe and North Africa. It was first recorded in North America in 1860 as an agricultural pest, and it is now found throughout the continent. The parasitoid wasp *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) is a gregarious endoparasitoid of several Pieridae (Laing & Levin, 1982). It was introduced into North America in 1883 to control *P. rapae* (Riley, 1885). Both the host and parasitoid are commonly found on wild and cultivated cruciferous host plants such as cabbage and wild mustard. *Cotesia glomerata* was the primary parasitoid of *P. rapae* in the study area. A tachinid parasitoid of *P. rapae* larvae was also found, rarely, in both habitat types. A hyperparasitoid of *Cotesia*, *Tetrastichus galactopus* (Hymenoptera: Eulophidae) was found in up to 30% of the late-season *C. glomerata* pupal clusters in both habitat types.

There are three to four generations of *C. glomerata* per season in the study area (S. van Nouhuys, unpubl. obs.), and wasps spend the winter in diapause as pupae in the leaf litter. Adults emerge in early spring to find *P. rapae* on early-flowering host plants such as *Barbarea vulgaris* (Root & Tahvanainen, 1969). The butterflies and wasps then move to other host plants as they become available. Adult wasps in the field feed on flower nectar, but how far they fly, how many hosts they parasitize, and how long they live are unknown. In the laboratory a female may lay eggs in six to eight hosts a day and live up to 2 weeks if well fed.

Wasp collection and field sites

The wasps used in this experiment came from four collection sites: two cabbage fields and two streamside, wild host plant populations. Both cabbage collection sites were near Geneva, NY, where cabbage has been the primary vegetable crop for many years. One was a 250 ha commercial field, and 10 km away was the second field at the New York State Agricultural Research farm, in which there are many small cabbage fields. Corn and other forage crops, and cabbage dominate the area between and surrounding the two cabbage sites. In an attempt to obtain a representative sample of the genetic variability present at each site,

parasitized host larvae were collected from widely dispersed plants in each field.

The two wild host plant collection sites were about 100 km away from the cabbage fields and 10 km distant from each other in Ithaca, NY. Throughout the season, host larvae were found on several different plant species in these sites: *Barbarea vulgaris*, *Brassica kaber* and *B. nigra*. These plants grow individually and in clumps among an array of herbaceous nonhost plants. There were no large-scale cultivated host plants within 30 km of these sites.

The density of host larvae in both habitats varied seasonally from 0 to 30 larvae per plant. Similarly, the fraction of hosts parasitized varied seasonally in both habitats from no parasitism to 75% of the larvae collected being parasitized. Although there was great temporal within-site variability of host density and fraction of larvae parasitized, the wild and cultivated habitats did not differ on average (S. van Nouhuys, unpubl. obs.).

Wasp rearing

We are interested in genetic differences between wasps from different sites when tested in different habitats. Therefore to standardize the rearing environment, the wasps used in the experiment were progeny of individuals collected from the four field sites. These progeny were grown in uniform laboratory conditions. The parasitized host caterpillars that we collected were reared in Petri dishes on collard leaves (*Brassica oleracea*, Acephala group) until wasp larvae emerged from the caterpillars to pupate. After pupation, each cluster of cocoons was put in a separate, clear plastic cup. Upon emergence, the adult wasps had access to honey and water. We allowed siblings to mate, as they are likely to do in the field (Tagawa & Hidaka, 1982), and then we removed individual females from the cups and let them parasitize second instar *P. rapae* larvae. These parasitized host larvae were reared separately in Petri dishes on collard leaves until wasps pupated, and then each cocoon cluster (of full- or half-sibs) was put in a cup with honey and water. Three-day-old sib-mated females from these cups were used in the experiment.

The host, Pieris rapae

The *P. rapae* larvae used for infestation of host plants came from a laboratory colony. To start this colony, adult butterflies were collected from early season host plant sites in both Ithaca, NY (four populations, 30 butterflies) and Geneva, NY (five populations, 26 butterflies), so that the host colony would not represent butterflies from any one habitat type. Adults fed on a solution of 10% honey in water, and the larvae were fed potted collard plants grown in a greenhouse.

To give the wasps oviposition experience before each observation without experience of a particular plant odour, *P. rapae* eggs laid on wax paper in the colony cages were transferred to a wheatgerm-based artificial diet containing no other plant material (Benrey, 1993). These larvae were maintained on artificial diet until exposure to wasps in a growth chamber at 24°C under a light regime of 16:8 h L:D.

Model habitats for testing behaviour

The test habitat cages were constructed out of wood and semitransparent mesh cloth and were housed in a greenhouse. A wood frame 1.68 m high supported the mesh cloth top and walls. On one side was a mesh door held closed with Velcro.

Each replicate cabbage habitat consisted of four cabbage plants (*B. oleracea* var. 'Gourmet'). Cabbage grown in the greenhouse were transplanted at 5-weeks-old into soil (originally from an agricultural field) in the base of each cage. The wild habitats were made by transplanting entire 1 m × 1 m plots of plants and soil from one of the wild host plant collection sites into the cages. In order to standardize the host plants in each replicate, the naturally occurring host plants (*B. kaber* and *Barbarea vulgaris*) were removed and replaced with four potted *B. kaber* in each cage. These *B. kaber* plants were grown in the greenhouse from seed collected from the same site during the previous year.

There were many species of nonhost plants in each wild test habitat cage. Although most of the same plant species appeared in each cage, some plants were unique, and the percentage cover by each plant varied. Although this inconsistency may introduce variation in wasp behaviour, it does not introduce a systematic bias into our results because wasps were randomized over the replicate cages. In order to standardize the feeding state of wasps during observations, we removed any flowers, and honey and water were made available in each caged habitat. None of the nonhost plants contained the volatile glucosinolate compounds attractive to *C. glomerata* (see van Nouhuys, 1997 for a list of plant species in each cage).

The host plants *B. kaber* and cabbage were of similar sizes. Ten to 12 leaves per plant were exposed on both host plant species. During the third week of the experiment the average exposed leaf area of cabbage was 287 cm^2 (SE 43 cm^2) per plant. The average exposed leaf area per *B. kaber* was 260 cm^2 (SE 68 cm^2). The difference in architecture between cabbage and *B. kaber* makes the actual areas available to a wasp or to a caterpillar difficult to compare.

Experimental design

Female progeny of wasps collected from each site were observed foraging in each test habitat type in a reciprocal transplant-style experiment (Fig. 1). To prepare the test habitats, each cage was infested with eight second-instar *P. rapae* larvae (two per host plant) 24 h before each trial. This is a realistic host density in both the wild and cultivated natural habitats. Larvae were placed on the top surface of opposite middle leaves. During the next 24 h, the larvae fed and moved to positions on the plant comparable to where second instar *P. rapae* larvae would be found on these plants in the field.

Two hours before each trial, individual 3-day-old mated, female wasps from a known population were placed in a vial with honey and water. One second-instar host larva, reared on artificial diet, was put in each wasp vial and was observed until the wasp had parasitized it. This oviposition experience was given in order to reduce the variability of behaviour, and to increase the probability of active foraging (Vet *et al*., 1990). In addition, this step eliminated the few individuals that were not motivated to parasitize, and reduced the possible effect of high egg load on behaviour (Mangel, 1989).

A wasp observation then proceeded as follows: a single wasp was placed on the top surface of a clean host plant leaf in a given cage. Five minutes were allowed for the wasp to become orientated to the environment, starting when it began to palpate its antennae. Some wasps immediately began to walk while palpating their antennae or to fly around the host plant, whereas others flew to the top of the cage and then back down into the plant canopy. They were then observed for 10 min. We recorded the wasps' behaviour by speaking into an audio-recorder during the observation, and then entering these data into a computer using event-recording software (Noldus, 1991). In order to measure the individual rate of parasitism, each wasp was then left in the host-infested test habitat for 6 h after observation. It was then removed, along with the host larvae, which were dissected 4 days later in order to count the number that had been parasitized. This permitted us to assay how successful a wasp with a given array of behaviours was at parasitizing hosts during a short observation.

Preliminary observations of *C. glomerata* behaviour using large portable cages were made in the field, along with observations of wild individuals encountered between 1992 and 1995. Based on these observations we are confident that the behaviours we observed in the caged model habitat that were associated with plants are similar to plant-associated behaviours seen in the field.

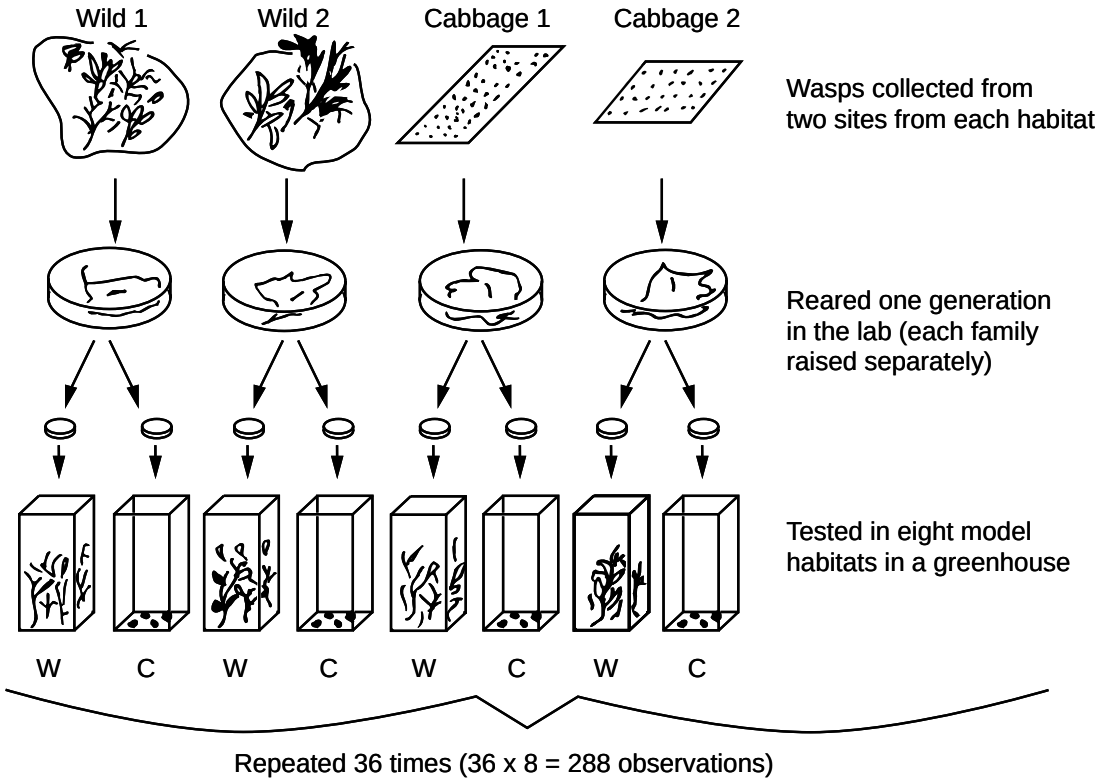

Fig. 1 A schematic drawing of the experimental design.

The behaviours associated with the cage wall, however, could not be observed in the field.

Each day, a single wasp from each of the four collection sites was observed in each test habitat type, for a total of eight wasps per day (Fig. 1). In an attempt to capture the genetic variability within each collection site, wasps from four different families were used for each trial. To reduce the experimental variance, one of a pair of sisters from each family was observed in cabbage and the other was observed in the wild test habitat. A new set of families was used in each trial. This was repeated 36 times from mid-July to mid-September of 1994, for a total of 288 wasps. The wasps were randomized among cages within each test habitat type. The order of wasp observation was also randomized within each trial, and the origin of the wasp being observed was coded so as to be unknown to the observer.

Analysis

Behavioural variables We recorded wasp location (host plant, cage wall or in the air around the host plant), type of locomotion (walking, flying or standing) and activity (including palpating antennae and grooming). These behaviours are expressed as the proportion of the total time for which each wasp was seen. The time during which a wasp was not visible (from 0 to 300 s, with a mean of 24 s) was subtracted from the total for each observation. The number of landings on each plant type, the number of movements between plants, and the number of times a host was encountered during the observation were also recorded.

The relative rate of individual parasitism was calculated as the fraction of available prey parasitized by an individual during 6 h, divided by the mean fraction parasitized in a given habitat. This is the estimate of relative fitness that we used in our analysis of natural selection.

MANOVA and how partitioning the data set allows different hypotheses to be tested Using multivariate analysis of variance (MANOVA) in SAS (PROC GLM, SAS Institute, 1989) we evaluated the effect of wasp source habitat, collection site nested in source habitat, test habitat type, and replicate cage nested in test habitat on a set of behavioural characters. We did not analyse variation among families within sites. A multivariate test of significance allows one to take into account all of the evidence for an association between factors (wasp source and test habitat) and a set of possibly correlated behaviours.

In order to test specific hypotheses about behaviour within each wasp source habitat and within test habitat types, we partitioned the data in two ways. This leads to the following five separate statistical models with which different hypotheses can be addressed (see Via, 1993 for another example of this type of partitioning).

The full model includes all of the wasps from both source habitats in both test habitat types. In this model the main effect of test habitat tests whether the 'average' wasp behaves differently in cabbage than it does in the wild test habitat. The main effect of wasp source tests for genetic differentiation between wasps from different source habitats in the 'average' test environment. The test habitat by wasp source interaction tests the hypothesis that the differences in behaviour seen in the two test habitats vary with source habitat.

The two source habitat models allow us to ask whether the cabbage and wild test habitats are different for wasps from a given source. The data are also partitioned by test habitat (test habitat models) so that the behaviour of wasps from the two sources can be compared within each of the two test habitats. The tests of significance of these MANOVA models allow us to ask whether there is genetic differentiation of behaviour between wasps from wild and cultivated sources when observed in either test habitat.

Canonical analysis Canonical analysis allows us to determine the contribution of each component of behaviour in a MANOVA model to differences between categories of a given factor. Each factor (source and test habitat) had two categories. Thus, if the canonical coefficient for a given behaviour is high, then that behaviour is likely to differ between categories within a factor. Canonical analysis has previously been used in ecology, systematics and psychology (see Gittins, 1985). Within evolutionary biology, canonical analysis has been used for the study of natural selection on the phenotype (e.g. Phillips & Arnold, 1989; Simms, 1990).

Using the canonical coefficients we found sets of behaviours that best differentiate between the cabbage and wild test habitats for each wasp source separately and the set that distinguishes between the behaviours of wasps from different sources in the cabbage test habitat. We were unable to find a suitable canonical model to distinguish between wasp sources in the wild test habitat. In each case, we started with many behavioural variables (20–25) and then systematically eliminated those that did not contribute to the MANOVA models, that is, that had canonical coefficients close to zero. After the model had been reduced to the point at which the removal of any more variables decreased the fit, behaviours that had been initially insignificant were individually added back in and they were kept if they significantly improved the model. This process was carried out separately for each of the partitioned data sets.

Because of the method used to eliminate variables, we cannot test for the statistical significance of the canonical coefficient for each behaviour in a model (James & McCulloch, 1990). Thus, the specific behaviours included in the models should not be interpreted as rigorous tests of hypotheses. The strength of this method lies in the fact that we are able to start with many variables, and through a relatively objective process of elimination, find a subset of variables that explains a large amount of the variability between groups.

Measurement of selection The behaviours and the rate of successful parasitism during the 6-h period immediately following the behavioural observation were measured individually for each wasp. These data were used to estimate the direct force of directional selection over the short-term on behavioural characters using partial linear regression (Lande & Arnold, 1983; Brodie & Janzen, 1995). Using this method, the relative individual fitness (relative rate of parasitism) is regressed on the set of behaviours in order to determine whether any of the characters influence variation in fitness. These are the characters under short-term natural selection.

The selection gradient (partial regression coefficient β) is the direct force of directional selection on a single character independent of the other measured traits. β is expressed in units of phenotypic standard deviations (Falconer, 1989), which allows comparison of selection on an array of characters regardless of their scale of measure. In order to reduce the problem of unmeasured characters for regression analysis (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987), we standardized the pre-observation experience of the wasps, because factors such as age, matedness and previous environment are likely to influence both behaviour and parasitism. Although we started with 20–25 behavioural variables, we removed highly correlated ones and those that did not contribute to the statistical models.

We measured the rate of parasitism for only 6 h. This was so that we could measure the fitness consequences of the behaviours that we observed. Although it would be ideal to measure the lifetime fitness, the rate of parasitism during one day early in life is certainly a component of fitness.

Using parasitism rate to test for local adaptation When populations within a species are found in several habitats there is the potential for local adaptation (higher fitness in the home habitats than seen in individuals from another habitat). We measured the parasitism rate of the progeny of wasps from both source habitats in both model habitat types and tested for local adaptation using a nested and crossed mixed-model analysis of variance with SAS PROC GLM (SAS Institute, 1989). A significant interaction between test habitat and wasp source in an analysis of variance of the parasitism rate would suggest that there is local adaptation of the rate of parasitism.

Results

Do wasps behave differently in the cabbage test habitat than in the wild test habitat?

Wasps behave differently while foraging in cabbage test habitats than while foraging in wild test habitats (effect of test habitat, Table 1). Thus, from the point of view of a wasp, regardless of its origin, the two test habitats are different. It is clear from the behaviours that distinguish between habitats that the presence of nonhost plants plays an important role in distinguishing the wild test habitat from the cabbage test habitat; for example, wasps stand on nonhost plants, hover around them, and even walk on nonhost plants while palpating their antennae (Table 1). Walking while palpating is generally thought of as active foraging behaviour (van Alphen & Vet, 1986). Note, however, that although wasps spend time on nonhost plants in the wild test habitat, it is not at the expense of spending time on the host plant. In fact, they walk on the host plant while palpating their antennae more on average in the wild habitat than in the cabbage test habitat (mean fraction of time in cabbage = 14% and mean in wild = 17%, $P < 0.05$). Not all variation in behaviour between the two habitat types is directly related to the presence of nonhost plants; for example, wasps spend less time on the top surface of the host leaves and groom more in cabbage than in the wild test habitat (Table 1).

Do wasps from cabbage behave differently than wasps from the wild host plant habitat?

In the cabbage test habitat, wasps from the cabbage source behaved differently from wasps from the wild source (Table 2, $P = 0.028$); for example, wasps from cabbage spent more time on cabbage and less time on the wall than wasps from the wild source habitat. Although wasps from both sources parasitized an equal number of hosts on average in cabbage (Fig. 2), wasps from cabbage were more likely to encounter a host during the observation than were wasps from the wild source (Table 2). We were unable to make a statistical model that distinguished between wasps from the wild and cultivated sources in the wild host plant habitat, which suggests that there is no significant differentiation of the behaviours expressed there.

Is there natural selection on wasp behaviour, and is it different in the cabbage from in the wild mustard test habitat?

Using regression to quantify the association between the parasitism rate and behavioural traits, we found that certain behaviours are associated with the rate of parasitism in each of the test habitats. We also found that different behaviours are associated with parasitism over a 6-h period in the two test habitats (Table 3). The only behaviour that appears to be under selection in both test habitats is antennae palpation. It is interesting to note that behaviours specific to nonhost plants do not seem to be under selection in the wild habitat

Table 1 Parasitoid wasp behaviour in cabbage and wild test habitats (wasp origin models). Behaviours are listed in the order of their contribution to canonical analysis distinguishing between habitat types in the MANOVA. The MANOVA models using these behavioural variables are significant at $P < 0.0001$. Date of observation (block) contributes significantly to the fit of both models. Replicate cage nested in test habitat type does not contribute significantly. The total canonical correlation (TCC) describes the fraction of variation of behaviours between treatment levels (test habitat) that is described by the MANOVA model

	Cabbage test habitat ($N = 131$ wasps)	Wild test habitat ($N = 133$ wasps)
Cabbage source wasps	*Don't* stand on nonhost plants	*Do* stand on nonhost plants
$F_{8,80}$(test habitat) = 20.27	*Don't* hover around nonhost plants	*Do* hover around nonhost plants
$P < 0.0001$	Walk on host plants while palpating *less*	Walk on host plants while palpating *more*
TCC = 0.82	On cage wall *less*	On cage wall *more*
	Stand on host plant *less*	Stand on host plant *more*
	Don't walk on nonhost plant palpating	*Do* walk on nonhost plant palpating
	Groom *more*	Groom *less*
	On top surface of leaf *less*	On top surface of leaf *more*
Wild source wasps	Walk on host plants while palpating *less*	Walk on host plants while palpating *more*
$F_{10,77}$(test habitat) = 16.10	*Don't* stand on nonhost plants	*Do* stand on nonhost plants
$P < 0.0001$	*Don't* hover around nonhost plants	*Do* hover around nonhost plants
TCC = 0.82	Groom *more*	Groom *less*
	Stand on host plant *less*	Stand on host plant *more*
	On top surface of leaf *less*	On top surface of leaf *more*
	Land on cage wall *less* frequently	Land on cage wall *more* frequently
	Don't land on nonhost plant	*Do* land on nonhost plant

Table 2 Behaviours that distinguish between wasp sources in the cabbage test habitat (test habitat model). The behavioural variables are listed in order of their contribution to the canonical analysis distinguishing between wasp sources in the MANOVA model. Date (block) contributes significantly to the fit of the model. Collection site nested in wasp origin does not contribute to the model. Multivariate analysis of variance is significant at $P < 0.03$, and the difference in behaviour between wasp sources is significant at $P = 0.028$ ($F_{10,82} = 2.17$). The total canonical correlation = 0.46, which means that 46% of the total variance between wasp sources is described by the model

Cabbage source waspss	Wild source wasps
Land on cabbage *less* frequently	Land on cabbage *more* frequently
On cabbage *more*	On cabbage *less*
Fly above plant canopy *less*	Fly above plant canopy *more*
On cage wall *less*	On cage wall *more*
Land on cage wall *less* frequently	Land on cage wall *more* frequently
Switch leaves *less* frequently	Switch leaves *more* frequently
Switch plants *less* frequently	Switch plants *more* frequently
Walk *less*	Walk *more*
On leaf bottom surface *more*	On leaf bottom surface *less*
Encounter prey during observation *more*	Encounter prey during observation *less*

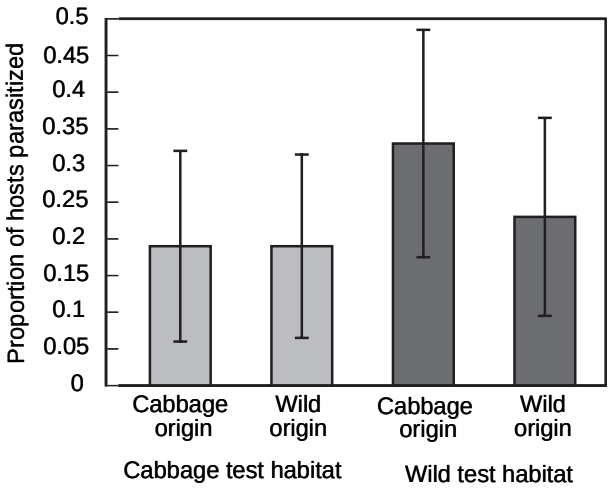

Fig. 2 The mean (and SE) rate of parasitism by wasps from both origins in each test habitat. In a crossed mixed-model analysis of variance there is a significant effect of wasp origin and of test habitat type (at $P < 0.05$). The statistical interaction between wasp origin and test habitat was not significant.

(Table 3b); for example, one might have assumed spending time on the nonhost plants to be negatively associated with rate of parasitism.

Are these wasps locally adapted?

Wasps did not parasitize more hosts in their home environment, providing no evidence that wasps have evolved to be locally adapted (Fig. 2). Although wasps from the wild origin had an equal parasitism rate in both test habitats, wasps from cabbage parasitized significantly more in the wild habitat than in cabbage.

Discussion

Are cabbage and wild test habitats different?

These two habitats are so different in plant composition and plant architecture that it is unlikely that a wasp would be able to behave the same in each habitat; for example, in the cabbage habitat, the only plant on which a wasp could land is a host plant, whether or not it is actively foraging. Although differences in behaviour caused directly by such characteristics of the habitat are not intrinsic to the wasp, they may have fitness consequences, and thus they are ecologically and evolutionarily important. There are also wasp behaviours associated with foraging that differ between habitats, such as time spent walking and palpating while on the host plant, which cannot be predicted simply by the physical differences between the test habitats.

The wasps do not just rest on nonhost plants between bouts of foraging; rather, they seem to forage actively by walking on the nonhost plants and hovering near the nonhost plant leaves. However, this behaviour does not seem to be at the expense of spending time doing the same activities on the host plants. In fact, wasps tend to walk on the host plant while palpating their antennae more in the wild test habitat than in cabbage. In addition, the average rate of parasitism in the wild habitat was higher (Fig. 2) than in cabbage. This contradicts the intuitive hypothesis that habitat

Table 3 The standardized direct selection gradients (β) for the regression of the relative parasitism rate on wasp behaviours while foraging in (a) the cabbage test habitat and (b) the wild test habitat. Gradients were calculated by a multiple regression of relative fitness on the set of behavioural characters

(a) Variables in cabbage regression model	β(SE)	(b) Variables in wild regression model	β(SE)
Duration palpate	0.22 (0.43)**	Duration standing	0.71 (0.67)**
Switch plants	0.30 (0.05)**	Duration fly within canopy	0.44 (0.56)**
Early encounter	0.23 (0.11)**	Duration palpating	0.57 (0.64)**
Frequency on wall	0.22 (0.06)*	Duration on plants	−0.50 (0.72)*
Duration walk not palpating	0.10 (0.59)	Duration on wall	−0.36 (0.90)*
Fly above plants	−0.15 (1.71)	Frequency on wall	0.25 (0.06)
Duration standing	0.12 (0.27)	Frequency on host plant	0.17 (0.04)
Switch leaves	0.14 (0.04)		
Frequency on host plant	−0.20 (0.03)		
$N = 129$ wasps		$N = 127$ wasps	
Multiple regression model fit	$P = 0.007$ $R^2 = 0.17$	Multiple regression model fit	$P = 0.08$ $R^2 = 0.13$

*$P < 0.10$, **$P < 0.05$.

complexity decreases foraging efficiency of individuals. Additionally, active foraging on nonhost plants, especially if it is not costly in terms of parasitism rate, may allow for the possibility of host and host plant range expansion.

Is there genetic differentiation of parasitoid wasp behaviour?

We found that wasps from the two source habitats have evolved to behave differently within the cabbage test habitat. In general, wasps from the wild sources moved more between plants and between the wall and the plants, spent more time on the wall of the cage and less time on cabbage than wasps from the cabbage origin. Wasps from the cabbage origin may have evolved to move less because host plants are close together in a cabbage foraging environment and the habitat patches are larger. Alternatively, wasps from cabbage may be more settled in the cabbage test habitat because it is similar to their home habitat, or the wasps may differ in their behavioural response to a caged environment. We found no significant difference in the multivariate behaviour of these two wasp populations in the wild test habitat.

Behaviours associated with rate of parasitism in cabbage are different from in the wild test habitat. Does this difference result from habitat complexity or from host plant species?

The behaviours associated with parasitism rate differ between the two test habitats (Table 3), suggesting that they are different selective environments. The few studies in which individual parasitoid foraging behaviour has been observed have found that complexity decreases parasitoid searching efficiency (e.g. Andow & Prokrym, 1990). However, we found no association between the amount of time wasps spent interacting with nonhost plants (one aspect of habitat complexity) and the parasitism rate. Also, overall parasitism rate in the wild test habitat was higher than in the cabbage test habitat (Fig. 2). Thus, the complexity in this system apparently does not decrease individual parasitoid success.

A second obvious difference between the two test habitats is host plant species. Response to host plant odour can depend on plant species, even among naive wasps (Geervliet *et al*., 1996; Benrey *et al*., 1997). However, higher parasitism in the wild host plant habitat cannot be attributed simply to preference for the odour of *B. kaber*, because flight response of *C. glomerata* to the odour of host-infested cabbage is greater than it is to *B. kaber* in a flight chamber (van Nouhuys, 1997; see also Benrey *et al*., 1997).

However, some aspects of the test environments must explain a higher rate of parasitism in the wild test habitat. Wasps seem to be more active in the wild test habitat, and thus they may parasitize more hosts. Alternatively, host plant architecture may be important; for example, the visual apparancy of feeding hosts may be greater on *B. kaber* than on cabbage, as feeding holes are visible on individual leaves because of the upright architecture of the plant. Wackers (1994) found the parasitoid *Cotesia rubecula* to be attracted to the sight of feeding damage, and Pimentel (1961) found *P. rapae* to be more vulnerable to *C. glomerata* on open-leafed varieties of *Brassica oleracea* than on closed-leafed *B. oleracea*.

Why is there no evidence for response to selection or local adaptation in cabbage?

If genetic differentiation of behaviour has evolved in response to natural selection in the two habitats, then one would expect to find that wasps from cabbage would exhibit more of the behaviours in cabbage that are favoured by selection than would wasps from the wild habitat. However, the observed genetic differentiation among wasps (Table 2) does not involve the characters that the selection analysis suggests are favoured (Table 3a). Thus, the pattern of selection that we detected in the cabbage test habitat does not necessarily reflect the ways in which the cabbage wasps have evolved. There is also no evidence of local adaptation because wasps were not more successful in their home test habitat. In fact wasps from cabbage parasitized more hosts, on average, in the wild habitat than they did in the cabbage habitat (Fig. 2). There are several possible explanations for this lack of local adaptation and response to selection.

1 *Local adaptation could be limited by gene flow*. Little is known about the dispersal of parasitoid wasps. However, the wild and cultivated source populations are 100 km apart so gene flow between them is probably low. We therefore do not have reason to believe that local adaptation is limited by gene flow.

2 *Genetic differentiation between populations could result from genetic drift under isolation by distance*. These wasp populations are not extremely small and so it is unlikely that drift would be a predominant driving force on the behaviours which are quantitative traits that are likely to affect fitness.

3 *Lack of genetic variability or correlation between behaviours related to fitness could constrain evolution*. Although there was sufficient phenotypic variability to detect selection on phenotypes, the extent of genetic variability of these characters is unknown. If there were little genetic variability of the characters that we found

were related to parasitism, then they would not evolve to be different. Unfortunately we have no information about the genetic variability of these characters. The behavioural characters under directional selection could be correlated in such a way that evolution would be constrained.

4 *The parasitism rate over 6 h in a caged habitat may not provide a complete measure of fitness*. An individual's fitness in an environment is the product of its behaviour, physiology, morphology and life history. Although the rate of parasitism over a 6-h period in a caged model habitat in the greenhouse is likely to be a component of fitness, it may not be a good predictor of the success of an individual wasp over a lifetime in the field. It has been shown that sometimes selection acting at different stages in the life cycle or caused by different adult fitness components (e.g. survival and reproduction) may favour different characters or even cause the same characters to be selected in different ways (Schluter *et al*., 1991).

Moreover, one aspect of the caged environment is very different from the situation that a wasp experiences in the field. Wasps in cages are unable to leave. Many of the behaviours that differed between wasp source habitats, and other behaviours found to be under selection, had something to do with the cage wall. Flying to the wall may well represent leaving the host plant patch. Because these wasps could not leave, most of them eventually returned to the plants. Thus, the characters found to be genetically differentiated between wasp source habitats may be under selection, but we may not have measured the components of fitness that caused the genetic differentiation. Despite this issue, we believe that the 6-h measurement of parasitism provides at least a partial view of the fitness consequences of the behaviours that we measured.

Conclusion

Many species are made up of populations that live in a variety of habitat types. The consequences of experiencing natural selection in different environments depend on both ecological and genetic factors. In order for natural selection to lead to the evolution of divergent populations, the habitats must differ in ways that affect fitness and there must be genetic variation of relevant traits. We have demonstrated the evolution of population divergence in behaviour for a parasitoid wasp that forages for one species of host in a variety of habitat types. Using test habitats modelled after the collection sites of the parasitoid wasps, we have also found evidence suggesting that a cabbage field may be a selective environment different from a stream-side wild host plant habitat. Although we did not show that the genetic differentiation observed was the result of selection in each environment, we did find the prerequisites for adaptive evolution in a complex ecological system. Had the experiment presented here involved only a few components of behaviour and one or a few aspects of the environment, the results may have been simpler to interpret, but ecologically and evolutionarily misleading. The results of this study may be a realistic illustration of the complexity of evolution of behaviour in natural systems in which many selective agents act upon each component of fitness, and where the constraints to evolution may differ between environments.

Acknowledgements

We would like to thank the City of Ithaca Department of Public Works, Hansen Farms, T. Shelton and M. Schmaedick for the use of field sites in Ithaca and Geneva, NY. For statistical assistance we appreciate the help of C. McCulloch and C. Olsen. I. Hanski, R. Hufbauer, S. Remold, A. Ruina and two anonymous reviewers made useful comments on previous versions of this manuscript. This research was supported in part by a National Science Foundation Graduate Research Fellowship, national and local chapters of Sigma Xi, and the Cornell University Department of Entomology Palmer Fellowship to S. van Nouhuys, and NSF DEB-9207573 to S. Via.

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