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Geographic variation in host acceptance by an insect parasitoid: genotype versus experience

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ABSTRACT

We compared host acceptance behaviour between two strains of the parasitoid *Cotesia glomerata*: one strain from the USA where *C. glomerata* was introduced from Europe 120 years ago, and one native European strain. In the USA larvae of *Pieris rapae* are attacked, whereas in Europe both *P. rapae* and *P. brassicae* serve as hosts. *Pieris brassicae* is the preferred host species, but since it is absent in the USA, it has not been available to American *C. glomerata* for about 350 generations. We observed clear geographic variation in host acceptance between American and European parasitoid strains: American *C. glomerata* rejected *P. brassicae* significantly more often than European parasitoids did. Early experience through development in and emergence from the less preferred host *P. rapae* increased acceptance of this host in European *C. glomerata*. Host acceptance of the preferred host was 'hardwired': it was high regardless of previous experience. Such strong inflexible responses to important stimuli and plastic responses to less important stimuli are observed in many other parasitoid–host systems. However, our results show that 350 generations of selection were sufficient to override this hardwiring in the American parasitoid strain.

Keywords: adaptation, behavioural differentiation, divergence, foraging decisions, intraspecific variation, invasion, learning, natural selection, phenotypic plasticity.

INTRODUCTION

Optimal foraging theory relates variation in the reproductive success of individuals to variation in their foraging behaviour. Several optimality models have focused on behavioural decision variables such as diet selection (e.g. MacArthur and Pianka, 1966; Stephens and Krebs, 1986). These classical static optimization models predict that optimal foragers rank the available prey items according to their profitability and only accept prey above a certain threshold profitability (e.g. Krebs and Davies, 1993; Godfray, 1994). The exclusion of lower-ranking prey is predicted to only depend on the encounter rate with

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higher-ranking prey. This modelling approach assumes that foragers have complete information on which prey species are currently available, and on the actual encounter rates in the environment. However, in natural systems the availability of top-ranking prey may be an unknown variable to naïve foragers. Information on the presence/absence of prey types and the respective encounter rates may have to be acquired through sampling and foraging experience (e.g. Stephens and Krebs, 1986). In this paper, we examine the acceptance of prey items by foragers from two different populations: one that is native to a complex environment that is unpredictable with respect to the presence/abundance of a top-ranking prey, and one that was introduced 120 years ago into a predictable environment where the top-ranking prey is always absent. We investigate genotypic differences between these populations after identical treatments and additionally investigate the effect of different types of acquired experience on subsequent acceptance of prey items.

The foragers used in our experiments are insect parasitoids. Female parasitoids forage for other insects to use these as hosts, a food source for their offspring. The larvae of many parasitoid species consume their host from the inside in order to complete development. Successful parasitoid development kills the host and allows adult parasitoids to emerge. This mode of reproduction causes a direct link between foraging behaviour and reproductive success in parasitoids, thus making them ideal model systems for evolutionary studies (van Alphen and Vet, 1986). Parasitoids are considered to be keystone species in many terrestrial ecosystems (LaSalle and Gauld, 1991, 1993), and food webs comprising green plants, insect herbivores and insect parasitoids include over half of all known animal species.

Interestingly, parasitoids are often introduced into a new environment by humans to suppress a population of a pest insect. Such classical biological control programmes can provide unique opportunities to test evolutionary hypotheses (Greathead, 1986; Le Masurier and Waage, 1993). The introduction of parasitoids in novel environments allows the comparison of traits between populations from the new and the original environment. Such comparisons after prolonged exposure to different selection regimes can provide information on the magnitude of evolutionary change in, for example, behavioural traits and on the mechanistic aspects of differentiation. One example of such a biological control project is the introduction of the parasitoid *Cotesia glomerata* (L.) in 1883–1884 (Clausen, 1956) from Britain to the USA to control the lepidopterous pest *Pieris rapae* (L.) that invaded North America from Europe and spread throughout Canada and the USA from the 1860s onwards (Chittenden, 1916; Clausen, 1956). Since its release, *C. glomerata* has also established itself within a few decades throughout most of North America.

In Europe, *C. glomerata* attacks the gregariously feeding larvae of the large white butterfly, *Pieris brassicae* (L.), the solitary larvae of the small white, *Pieris rapae*, and to a lesser extent *Pieris napi*, in which field survival is low (Geervliet *et al.*, 2000). In North America, *P. brassicae* is absent and *P. rapae* the main host, while *P. napi* (L.) may be attacked (R.G. Van Driesche, personal communication). Both *P. brassicae* and *P. rapae* larvae are suitable for *C. glomerata* development, especially the first three instars (Brodeur *et al.*, 1998). *Pieris brassicae* is the preferred host species when parasitoids are given a choice (E.A. van der Grift, M.P. Huijser and L.E.M. Vet, unpublished). *Pieris brassicae* sustains the development of more females (Brodeur *et al.*, 1998) and of larger clutches of larger wasps in a single larva (Harvey, 2000). This host occurs in clusters of up to 150 larvae, representing a 'jackpot' package of resources for the development of parasitoid offspring. *Cotesia glomerata* female fecundity varies between 500 and 2200 eggs (Moiseeva, 1976; Shapiro, 1976; Laing and Levin, 1982; Le Masurier and Waage, 1993; Tagawa, 2000) that are laid in clutches of about 20 eggs per host. In Europe, *C. glomerata* may experience seasons or years in which clusters of *P. brassicae* are extremely rare, while they can be abundant in others (Pak *et al.*, 1989). This means that European *C. glomerata* may have a choice between *P. brassicae* and *P. rapae* in some years or seasons, but cannot count on *P. brassicae* to be always available. What are the mechanisms *C. glomerata* uses to determine whether it accepts the less preferred larvae of *P. rapae*, which it is more likely to encounter regularly, or reject these as a host to spare its eggs for a possible 'jackpot' cluster of *P. brassicae* larvae? It may not be adaptive to use a fixed decision rule like 'only accept *P. brassicae*'. Instead, female parasitoids may use various cues for the presence or absence of *P. brassicae* in the current environment. For example, parasitoids may develop in either *P. brassicae* or *P. rapae* and memorize characteristics of the host odour that is present on their cocoon upon emergence (see Hérard *et al.*, 1988). Alternatively, they may use oviposition experience with *P. brassicae* as a cue to start rejecting *P. rapae*, or learn to use subtle differences in plant volatile blends from *P. brassicae*- versus *P. rapae*-infested plants (Geervliet *et al.*, 1998), and subsequently only accept larvae on plants with *P. brassicae* damage. A combination of such cues could also be used.

In Europe, the percentage parasitism achieved by *C. glomerata* in the field is usually higher for *P. brassicae* than for *P. rapae* (Moss, 1933; Richards, 1940; Sengonca and Peters, 1991; Geervliet *et al.*, 2000). Because of the absence of *P. brassicae* in North America, natural selection can be expected to have resulted in adaptation of the introduced *C. glomerata* population to foraging for the solitary host *P. rapae* (Le Masurier and Waage, 1993). Recent studies have found substantial differences in spatial foraging behaviour in European and North American populations of *C. glomerata* (Vos, 2001; Vos and Hemerik, 2003; Vos *et al.*, in press). In addition, an individual-based model study showed that the American foraging strategy results in a higher lifetime reproductive success on a Poisson distribution of *P. rapae* larvae over plants than the European foraging strategy (Vos and Hemerik, 2003). The North American parasitoids show a striking shift in the relative expression of different foraging behaviours. They exhibit much less explorative flights and hovering behaviour, traits that are necessary for the location of highly clustered *P. brassicae* larvae (Vos *et al.*, unpublished; see also Wiskerke and Vet, 1994; Vos *et al.*, 1998). This reduction of spatial mobility in American *C. glomerata* saves time and energy that is now allocated to the exploitation of patches with *P. rapae* (Vos, 2001; Vos and Hemerik, 2003; Vos *et al.*, unpublished). Le Masurier and Waage (1993) showed that more than 100 years after their introduction, *C. glomerata* from the USA have a higher attack rate on *P. rapae* than British *C. glomerata* (in the laboratory). Their experiment consisted of confining the hosts with a parasitoid for 2 h in a cage, after which the larvae were dissected to determine levels of parasitism. The results from this 'black box' experiment were in agreement with the prediction that natural selection has changed the behaviour of *C. glomerata* in its new environment, where it attacks *P. rapae* in the absence of *P. brassicae*. However, these results did not provide any clue as to which aspects of parasitoid behaviour might be different between American and British strains of *C. glomerata*. Moreover, the American parasitoids used in the experiments of Le Masurier and Waage (1993) had been reared on *P. rapae*, whereas the British parasitoids were cultured on *P. brassicae*. The different environments experienced by immature parasitoids during development, and the different stimuli available to newly emerged parasitoids in early adulthood, may have affected the outcome of these experiments (Le Masurier and Waage, 1993).

One important aspect of parasitoid behaviour that may affect attack rates is host acceptance. Differences in host acceptance between the different strains of *C. glomerata* could explain the higher rate of attack as observed by Le Masurier and Waage (1993). We compared host acceptance between American and European *C. glomerata* populations to determine whether the host species in which parasitoids develop has a phenotypic effect on host acceptance in the adult parasitoids. In addition, we examined how European *C. glomerata* use foraging experiences with either *P. rapae* or *P. brassicae* to make subsequent decisions on host acceptance. Specifically, we addressed the following questions: (1) Do American and European strains of the parasitoid *C. glomerata* differ in their host acceptance of *Pieris rapae* and *P. brassicae*? (2) Do European *C. glomerata* parasitoids show phenotypic differences in host acceptance after development in different host species (for one generation)? (3) Do European *C. glomerata* use oviposition experiences in *P. brassicae* versus *P. rapae* as a cue to change subsequent host acceptance? (4) Do geographic origin and developmental/early adult experience affect *C. glomerata*'s clutch size in *P. rapae*? (5) Can we understand the results in the light of the different ecological environments in Europe and North America? In this paper, we show significant differences in host acceptance behaviour between European and North American strains of the parasitoid *C. glomerata*, about 350 generations after *C. glomerata*'s introduction from Europe to North America. We discuss our results on strains from the Netherlands and USA in the light of published results on other strains from the USA, UK and Switzerland.

MATERIALS AND METHODS

The food plants were 3-month-old Brussels sprout plants (*Brassica oleracea* L. var. gemmifera cv. Icarus), grown in a greenhouse (20–25°C, 50–70% relative humidity, L16 : D8). The host species were the small white *P. rapae* and the large white *P. brassicae*, which originated from field-collected individuals from the vicinity of Wageningen, The Netherlands, and were reared in a climate chamber (22–24°C, 40–70% relative humidity, L16 : D8). The preferred first instar larvae of both host species were used in the experiments. The European parasitoid culture was established from *P. brassicae* larvae that had been placed in, and were recaptured from, Brussels sprout fields near Wageningen, the Netherlands, during the summer of 1998. This collection yielded about 100 cocoon clusters (2000 parasitoids). This European strain of *C. glomerata* was continually reared on first instar larvae of *P. brassicae*, in a greenhouse compartment (21–25°C, 50–70% relative humidity, L16 : D8). Parasitoid cocoons were stored at 12–13°C; emerged parasitoids were kept in (40 × 30 × 30 cm) cages (23°C, 50–70% relative humidity, L16 : D8). Ample water and honey were present. Each generation of parasitoids from the above strain was also allowed to oviposit in the alternative host, *P. rapae*. Thus we also had *C. glomerata* at our disposal that were reared on *P. rapae* for one generation, under the same conditions as described above.

The American strain of *C. glomerata* was established from 32 cocoon clusters, about 600 individuals, collected from fifth instar *P. rapae* from a white cabbage *Brassica oleracea* L. field in Geneva, New York, USA, and kindly sent to us by Mark Schmaedick and Tony Shelton in August 1997. The American *C. glomerata* parasitoids were continuously reared on Dutch *P. rapae* under the same conditions as the European strain. Both the European and the American parasitoid strains had been reared in the laboratory for several generations before being used in the experiments.

Experiment 1

The experiments were carried out in the laboratory at 23–24°C, 40–50% relative humidity and about 6000 lux. For the experiments, 3- to 5-day-old mated female parasitoids were collected from the culture cages and individually held in a vial with wet cotton wool and a honey droplet. These parasitoids did not have prior experience with hosts or plant materials. They only had experience with the cocoons they emerged from. Just before an experiment, pieces of leaf (0.5–0.7 cm^2) containing a single host, either *P. rapae* or *P. brassicae*, and its feeding damage were cut out of infested Brussels sprout leaves. The leaf disc was placed in the vial, where the parasitoid was allowed to walk onto it and search for the host. For each replicate a new parasitoid, vial and leaf disc were used. A parasitoid's first contact with the host occurred through antennation, a short external stab of the ovipositor, or by touching the host with the mouth parts. We recorded whether first contact was followed by an insertion of the ovipositor into the host. Hereafter we will refer to this phase in behaviour as *antennal acceptance*. Walking away after first contact was recorded as *antennal rejection*. After an ovipositor insertion, each larva was dissected in water under a microscope, with a light source underneath the dissection slide, in order to count the number of parasitoid eggs present inside. When no eggs were observed, this was scored as *ovipositorial rejection*. When one or more eggs were found, this was recorded as *ovipositorial acceptance*, which is host acceptance.

Six different treatments were used: European *C. glomerata* reared on *P. brassicae* or reared on *P. rapae* and American *C. glomerata* reared on *P. rapae* were tested on either *P. rapae* or *P. brassicae*. Thus it was possible to separate phenotypic effects (by development in different host species or early adult experience with cocoons) from genotypic effects (European versus American strains) on host acceptance. Each of the six treatments was replicated 36 times. All these 216 parasitoids made a first contact with a host larva.

Experiment 2

In Experiment 2a, we repeated part of Experiment 1, several months later: we tested for host acceptance of *P. rapae* by European *C. glomerata* reared on *P. brassicae* or reared on *P. rapae* and by American *C. glomerata* reared on *P. rapae*. In Experiment 2b, we measured the effect of a series of oviposition experiences with *P. rapae* versus *P. brassicae* on subsequent acceptance of *P. rapae* as a host, using European *C. glomerata* reared on *P. rapae*. The experimental procedure was similar to that of Experiment 1. Each parasitoid was allowed to walk onto a leaf disc that contained a single *P. rapae* or *P. brassicae* larva, until it attacked the larva by inserting its ovipositor. This was repeated twice more within about 30 min, each time with a new leaf disc and larva. After this treatment, each parasitoid had attacked three larvae of a single host species. Dissections of part of these larvae showed that almost all of such attacks resulted in actual ovipositions. Subsequently, acceptance of *P. rapae* by these European *C. glomerata* was examined, as in Experiment 1. Results from Experiment 1 showed a similar pattern for host acceptance at the antennal and ovipositorial level. In Experiment 2, we only observed antennal acceptance/rejection. For each treatment, 30 replicates were performed.

Statistical analyses

Chi-square tests for 2×2 tables with Yates correction for continuity were used for host acceptance data. As some groups were used in a number of comparisons (n), differences were considered significant at P-values below $0.05/(n)$, the so-called Bonferroni correction. Clutch sizes were compared with a multifactorial analysis of variance. The three qualitative factors were: geographical origin (USA or Europe), experimental host (*P. rapae* or *P. brassicae*) and developmental host (*P. rapae* or *P. brassicae*). Clutch sizes following different oviposition experiences were compared using a Mann-Whitney U-test.

RESULTS

Experiment 1

Variation in host acceptance between European and North American parasitoids

American *C. glomerata* that were reared on *P. rapae* rejected *P. brassicae* significantly more often than European *C. glomerata* that were reared on *P. rapae*, indicating differentiation in the acceptance of *P. brassicae* between the two parasitoid populations ($\chi^2_1 = 20.01$, $P \ll 0.001$; Table 1). The proportion of *P. brassicae* larvae accepted by the European parasitoids was 0.972, compared to only 0.472 in the North American parasitoids.

Genotype versus early experience with the natal host

European *C. glomerata* that were reared on their preferred host, *P. brassicae*, rejected *P. rapae* significantly more often than parasitoids of the same population that had been reared on *P. rapae* for one generation ($\chi^2_1 = 8.31$, $P = 0.0039$; Table 2). European *C. glomerata* reared on *P. brassicae* also rejected *P. rapae* significantly more often than American *C. glomerata* reared on *P. rapae* ($\chi^2_1 = 13.55$, $P < 0.001$). (*Note*: this is the same situation as in the experiment by Le Masurier and Waage (1993).)

Most of the European *C. glomerata* parasitoids accepted *P. brassicae* for oviposition. There was no difference in acceptance of this preferred host due to early experience with the rearing host ($\chi^2_1 = 0.26$, non-significant; Table 1). Most of the European and American *C. glomerata* parasitoids that were reared on *P. rapae* accepted this species as a host (Table 2).

Table 1. Host acceptance of *P. brassicae*, by *C. glomerata* parasitoids from Europe (EUR) and North America (USA), after development in *P. brassicae* or *P. rapae*

Strain origin, natal host	Antennal rejection	Antennal acceptance	Ovipositorial rejection	Ovipositorial acceptance	Total rejection	Total acceptance
EUR, *P. brass.*	0	36	3	33	3	33 (0.917)^a
EUR, *P. rapae*	0	36	1	35	1	35 (0.972)^a
USA, *P. rapae*	12	24	7	17	19	17 (0.472)^b

Note: Different superscripts (a, b) indicate significant differences in (total) host acceptance. Note that the US strain has not had access to *P. brassicae* for about 350 generations.

Table 2. Host acceptance of *P. rapae*, by *C. glomerata* parasitoids from Europe (EUR) and North America (USA), after development in *P. brassicae* or *P. rapae* (Experiment 1)

Strain origin, natal host	Antennal rejection	Antennal acceptance	Ovipositorial rejection	Ovipositorial acceptance	Total rejection	Total acceptance
EUR, *P. brass.*	10	26	11	15	21	15 (0.417)^a
EUR, *P. rapae*	2	34	6	28	8	28 (0.778)^b
USA, *P. rapae*	0	36	5	31	5	31 (0.861)^b

Note: Different superscripts (*a*, *b*) indicate significant differences in (total) host acceptance.

So there was no difference in host acceptance of *P. rapae* between the European and American strains, when they had been reared on the same host, *P. rapae* ($\chi^2_1 = 0.38$, $P = 0.54$; Table 2).

Theoretically, parasitoids might show a similar acceptance of hosts for oviposition, but still show a differential allocation of eggs to larvae of the different host species. However, such an effect was not observed. Neither the geographical origin of the parasitoid strain, nor the experimental host species or the developmental host species, had a significant effect on clutch size (multifactorial ANOVA: $F_{1,\text{geographical origin}} = 2.12$, $P = 0.15$; $F_{1,\text{experimental host}} = 0.56$, $P = 0.47$; $F_{1,\text{developmental host}} = 0.008$, $P = 0.93$) (Fig. 1).

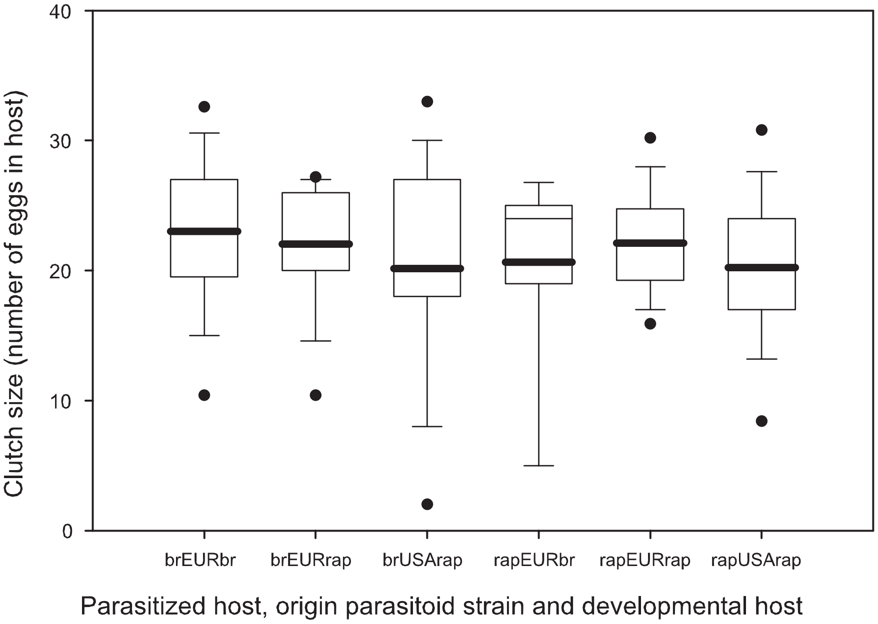

Fig. 1. Mean clutch sizes in the hosts *P. brassicae* and *P. rapae* by *C. glomerata* parasitoid strains from Europe (EUR) and North America (USA), which had developed in *P. brassicae* (br) or *P. rapae* (rap). Error bars represent standard deviations.

Experiment 2

Genotype versus early experience with the natal host

In Experiment 2a, we repeated the three treatments of Experiment 1 where acceptance was tested on the host *P. rapae*. This yielded essentially the same results: European *C. glomerata* rejected *P. rapae* more often after development in *P. brassicae* ($\chi^2_1 = 7.33$, $P = 0.0068$; Table 3), while there was no difference between American and European parasitoids when both had developed in *P. rapae* ($\chi^2_1 = 0.14$, $P = 0.70$; Table 3).

Oviposition experience as a cue?

The results of Experiment 2b showed that 27 of 30 *C. glomerata* parasitoids antennally accepted *P. rapae* after three ovipositions in *P. rapae* (and development in *P. rapae*). Surprisingly, antennal acceptance of *P. rapae* still occurred 25 out of 30 times after three ovipositions in *P. brassicae* (Table 4). Thus a series of oviposition experiences in the preferred versus less preferred host did not significantly affect subsequent acceptance of *P. rapae* in a no-choice situation ($\chi^2_1 = 0.14$, $P = 0.70$). The average clutch size in *P. rapae* after a series of ovipositions in *P. brassicae* (16.6, $N_{\text{dissected}} = 17$) did not differ significantly from the average clutch size after a series of ovipositions in *P. rapae* (16.3, $N_{\text{dissected}} = 12$; Mann-Whitney U-test, $Z = -0.089$, $P = 0.93$). The average first clutch size was 24.3 eggs in this experiment. Clutch size decreased to about 16.5 eggs after a series of three ovipositions.

Table 3. Host acceptance of *P. rapae*, by *C. glomerata* parasitoids from Europe (EUR) and North America (USA), after development in *P. brassicae* or *P. rapae* (Experiment 2)

Strain origin, natal host	Antennal rejection	Antennal acceptance
EUR, *P. brassicae*	16	14 (0.467)^a
EUR, *P. rapae*	5	25 (0.833)^b
USA, *P. rapae*	3	27 (0.900)^b

Note: Different superscripts (*a*, *b*) indicate significant differences in antennal host acceptance.

Table 4. Host acceptance of *P. rapae*, by *C. glomerata* parasitoids from Europe (EUR), after three oviposition experiences with either *P. rapae* or *P. brassicae*

Strain origin, natal host	3 oviposition experiences with:	Antennal rejection	Antennal acceptance
EUR, *P. rapae*	*P. rapae*	3	27 (0.900)^a
EUR, *P. rapae*	*P. brassicae*	5	25 (0.833)^a

Note: Identical superscript (*a*) indicates no significant difference in antennal host acceptance.

DISCUSSION

Evolution since Darwin?

In 1883, the year after Darwin died, the parasitoid *Cotesia glomerata* was shipped from Britain to North America. It was released to control the invasive insect *Pieris rapae*, became established in 1884 and has foraged in its new environment ever since. Thus *C. glomerata* became part of an unintended evolutionary field experiment (Le Masurier and Waage, 1993). In the present study, we found significant differences in host acceptance behaviour between European and North American *C. glomerata* populations, about 350 generations after *C. glomerata*'s introduction to America. A major difference between the original and new environment is the consistent absence of the highly profitable host *P. brassicae* in North America, and its presence at unpredictable densities in Europe (including Britain). This difference in ecological conditions led us to compare host acceptance between the two parasitoid strains and to investigate the plasticity of European *C. glomerata* behaviour in response to information on the presence of the top-ranking host.

Why do American *C. glomerata* parasitoids reject highly profitable *P. brassicae*?

We showed that 97% of European parasitoids accepted *P. brassicae* as a host, while the majority of American parasitoids rejected *P. brassicae* (both strains reared on *P. rapae*). This raises the question of why American *C. glomerata* reject this highly profitable host. One might argue that there may be no costs involved in maintaining an unexpressed preference for a rich resource. However, this may not be true. Rejection of *P. rapae* and acceptance of *P. brassicae* may be correlated characters in a single behavioural repertoire: parasitoids that tend to reject *P. rapae* in Europe may be rewarded for holding on to their eggs when they encounter a cluster of *P. brassicae*. In the North American environment, even a slight tendency to reject *P. rapae* will be strongly selected against, and consequently a high tendency to accept *P. brassicae* may be lost simultaneously. It is also possible that a correlation exists between spatial foraging traits that are adapted to the highly clustered spatial distribution of *P. brassicae* and the tendency to accept this species as a host. Recent studies have shown that two populations of American *C. glomerata* (from New York State and Massachusetts) spend almost no time on the explorative flights and extensive hovering that are so characteristic of their European conspecifics. There are no significant differences in six different traits for spatial foraging behaviour between the New York and Massachusetts strains (Vos, 2001; Vos *et al.*, unpublished). In both of these populations, spatial mobility is greatly reduced relative to that of European parasitoids. This effectively allows American *C. glomerata* to allocate more time and energy to host-searching behaviour on *P. rapae* patches (Vos, 2001; Vos and Hemerik, 2003). It is quite conceivable that explorative traits for the location of rare *P. brassicae* clusters and traits for the acceptance of this host are genetically correlated. The absence of this host in North America may have selected for a loss or decreased expression of these traits within the entire behavioural repertoire.

Variation within and between continents

The present analysis is based on two contemporary populations. No data are available on host acceptance in the British parasitoids that were originally introduced to North America, in 1883. It is a good question to what extent the tested European and North American populations are representative of other *C. glomerata* populations on these continents. To interpret our results in relation to behavioural variation *within* continents, a comparison between contemporary British and Dutch *C. glomerata* is especially relevant. The available evidence shows that these strains are more similar to each other than to the North American strains. Le Masurier and Waage (1993) showed that American *C. glomerata* parasitoids cultured on the host *P. rapae* parasitized *P. rapae* larvae at a significantly higher rate than British *C. glomerata* cultured on *P. brassicae*. We could repeat this result using a different American *C. glomerata* strain and a Dutch *C. glomerata* strain: these American parasitoids cultured on the host *P. rapae* parasitized *P. rapae* larvae at a significantly higher rate than Dutch *C. glomerata* cultured on *P. brassicae* (Table 2).

Furthermore, Le Masurier (1990) observed British *C. glomerata* cultured on *P. brassicae* to readily accept *P. brassicae* for oviposition (93%, $n = 30$). This is very similar to the results obtained in the present study, as we observed Dutch *C. glomerata* cultured on *P. brassicae* to also readily accept *P. brassicae* for oviposition (92%, $n = 36$). In contrast, we found that American *C. glomerata* cultured on *P. rapae* did not readily accept *P. brassicae* (the European host) any more (47%, $n = 36$). By also rearing the Dutch strain on *P. rapae*, we could show that the reduced acceptance of *P. brassciae* by American *C. glomerata* was not a developmental host effect. This became clear with the Dutch strain cultured on *P. rapae* still readily accepting *P. brassicae* as a host (97%, $n = 36$). *Pieris brassicae* is of higher quality to *C. glomerata* than any other *Pieris* species in Europe (Brodeur *et al.*, 1998; Harvey, 2000). There is no empirical or theoretical argument why other European *C. glomerata* populations should have a low acceptance of this top-ranking host, and as mentioned above the interesting issue is why North American *C. glomerata* do not readily accept it anymore.

With respect to behavioural variation within and between continents, most is known about spatial foraging behaviour. We do know that spatial orientation and flight behaviour are very similar in two European *C. glomerata* populations from the Netherlands and Switzerland (H. Gu, personal communication; see also Gu and Dorn, 2000). This 'European' behaviour differs dramatically from the spatial foraging in two North American parasitoid populations from New York State and Massachusetts (Vos, 2001; Vos *et al.*, unpublished). Many aspects of spatial foraging behaviour are indistinguishable in these two American strains, but are significantly different from the Dutch strain (Vos, 2001; Vos *et al.*, unpublished). In conclusion, all the available evidence shows larger behavioural differences between than within continents.

A potential evolutionary scenario

We have shown that European *C. glomerata* have a high tendency to reject *P. rapae*, after development in *P. brassicae*. This implies that the first generation of *C. glomerata* in the USA may have been relatively unsuccessful. However, we have also shown that only a single generation of development in *P. rapae* is sufficient to dramatically increase the acceptance of this host in European *C. glomerata*. This means that the first hurdle in the novel environment was probably quickly overcome. This result on the effect of the natal host suggests that phenotypic plasticity in acceptance of a less preferred host may have quickly paved the way for subsequent genetic change, for example in terms of foraging adaptations to the spatial distribution of *P. rapae* instead of *P. brassicae*. A loss of traits for foraging on clustered *P. brassicae* may have been associated with a loss of genetically correlated traits for a high potential acceptance of this host.

Why does oviposition experience with *P. brassicae* fail to induce rejection of *P. rapae*?

We showed that three ovipositions by European *C. glomerata* in the preferred host *P. brassicae* did not lead to increased subsequent rejections of *P. rapae*. This makes us question whether ovipositions have a high informational value under field conditions. Using a series of ovipositions as a cue for the presence of *P. brassicae* in the environment is a bit of an *a posteriori* way of dealing with information: If *C. glomerata* lands on a large cluster of *P. brassicae*, it can deposit its entire egg complement in these preferred hosts. Learning to reject *P. rapae* would become rather irrelevant after such an event. So, oviposition experiences may not be a useful source of information for parasitoids that have to decide whether or not to accept the many *P. rapae* larvae they will most likely encounter before ever coming upon a cluster of their preferred host species. Maybe the only *a priori* information available to a parasitoid comes from the natal host: if odours on a parasitoid's cocoon indicate development in *P. brassicae*, that host may still exist as a population in the environment. We have indeed observed that development in *P. brassicae* led to a significantly higher rejection of *P. rapae*.

A comparison with the results of Le Masurier and Waage (1993)

European *C. glomerata* rejected *P. rapae* significantly more often after development in *P. brassicae* than after having *P. rapae* as its natal host. This phenotypic effect of the developmental host may have played a role in the experiments of Le Masurier and Waage (1993), and may explain the observed difference in attack rates between British and American *C. glomerata*: British *C. glomerata* may have had a significantly lower attack rate on *P. rapae* due to their development in *P. brassicae*.

Currently, we know that the spatial aspects of foraging behaviour are also important in determining attack rates and lifetime reproductive success in the European and North American strains (Vos and Hemerik, 2003). The cage experiment by Le Masurier and Waage (1993) provided valuable information, but was not designed to allow a full expression of the spatial foraging repertoire of these parasitoids.

Clutch size decisions

Potentially, parasitoids might not only accept or reject a host. They could also decide to insert larger clutches of eggs in some host species than in others. We did not find any difference in clutch sizes between parasitoids of different geographical origins or from different rearing hosts. Oviposition experiences with different hosts did not significantly affect clutch size either. Some conflicting evidence exists on clutch size allocation in *C. glomerata*. Brodeur *et al.* (1998) showed that European *C. glomerata* (reared on *P. brassicae*) produced larger clutch sizes on *P. brassicae* than on *P. rapae*. The parasitoids emerging from these larger clutches in *P. brassicae* did not differ in any life-history parameter from those emerging from the smaller clutches in *P. rapae*, which confirms *P. brassicae*'s role as a better host for *C. glomerata*. Harvey (2000) observed that European *C. glomerata* allocated larger clutches to *P. brassicae* than to *P. rapae*, and showed that wasps from *P. brassicae* tended to be larger. However, Brodeur and Vet (1995) did not find a difference in *C. glomerata* clutch size in *P. rapae* versus *P. brassicae*. Moreover, Le Masurier (1991) found the opposite, a higher clutch size of British *C. glomerata* (reared on *P. brassicae*) in *P. rapae* than in *P. brassicae*. Le Masurier did not observe a difference in clutch size between American and British *C. glomerata* in *P. rapae*. The above studies and our experimental results suggest that *C. glomerata* clutch sizes are highly contingent on the experimental set-up, procedure and state of the animals, and that clutch size is apparently not a major factor in adaptation of *C. glomerata* to the North American environment.

Parasitoid fecundity after development in *P. rapae* versus *P. brassicae*

Preliminary experiments did not show any difference in parasitoid development time or survival in *P. rapae* and *P. brassicae* between the American and European strains (M. Vos, unpublished). However, it is likely that *C. glomerata* has a considerably larger fecundity after development in *P. brassicae* than in *P. rapae*: Le Masurier and Waage (1993) found that British *C. glomerata* emerged with about 800 eggs after development in *P. brassicae*, which was considerably more than the 500 eggs in American *C. glomerata* that had *P. rapae* as their natal host. However, to rule out a possible genotypic effect, fecundity should also be measured in American *C. glomerata* reared on *P. brassicae*.

Parasitoid population differences and the developmental host

Host acceptance has been studied extensively in parasitoid species that are used for biological control purposes (e.g. Bornstein and Hoffmann, 1995, 1998; Hunter *et al.*, 1996; Rojas *et al.*, 1999). Often these parasitoids are reared on a factitious host that can be cultured more easily, but would not be found or parasitized in the field. Continuous rearing on a single host species may result in a micro-evolutionary ‘experiment’: the parasitoid strain may undergo selection pressures that tune it into using that host, possibly at a cost in traits for dealing with the target host in the field.

A strain of the parasitoid *Catolaccus grandis* showed a significant switch in host acceptance after only 10 generations of rearing on the factitious host *Callosobruchus maculatus*. This could not be reversed by rearing this parasitoid for a single generation on its natural host *Anthonomus grandis* (Rojas *et al.*, 1999). A population of the parasitoid *Encarsia formosa* that had been reared for many years on the host *Trialeurodes vaporariorum* accepted a lower percentage of *Bemisia tabaci* whitefly hosts compared to a population that had been reared for several years on *B. tabaci* (Hunter *et al.*, 1996). It is also possible that many of the foraging characteristics of the field population are retained, while the acceptance of the laboratory strain increases. A *Trichogramma minutum* strain that was collected from oblique-banded leaf roller eggs (*Choristoneura rosaceana*) had a much higher level of acceptance of this host than several *T. minutum* strains collected from other hosts. However, all parasitoid strains had a high level of acceptance of the rearing host *Ephestia kuehniella* (McGregor *et al.*, 1998).

Bornstein and Hoffmann (1998) found that parasitoid experience of the rearing host or ovipositions altered the acceptance of low-ranked hosts, but not of highly preferred hosts, in four strains of *Trichogramma brassicae*. A similar effect was observed in the parasitoid *Diachasmimorpha tryoni*: experience did affect host responses on less-preferred hosts but not on preferred hosts (Duan and Messing, 1999). This effect on the acceptance of only low-ranked hosts was similar in our European *Cotesia–Pieris* system: experience of the rearing host did affect acceptance of the less preferred host *P. rapae* but not of the preferred host *P. brassicae*. Apparently, acceptance of a high-quality host is to some extent 'hardwired' in these systems, while acceptance of a less preferred host is flexible and can serve as a 'back-up' under conditions where the best host is not available. Such flexibility may also facilitate invasions into new environments.

Despite the fact that acceptance of *P. brassicae* is hardwired in European *C. glomerata*, the American parasitoids do not readily accept *P. brassicae* as a host. Traits for such an immediate acceptance may have been lost during the 350 generations *C. glomerata* did not have access to *P. brassicae* in North America. Behavioural differentiation between geographically separate populations often seems to occur by the loss of (parts of) behaviour patterns, or by changes in the frequency of their expression (Foster, 1999). Crosses between populations of different geographic origins could illuminate the genetic contribution to variation in traits like host acceptance (Hopper *et al.*, 1993) and lead to fundamental insights in behavioural differentiation and local adaptation.

CONCLUSIONS

Classical biological control projects and invasions of species in new environments provide challenging opportunities to study the processes that lead to genetic divergence between populations. Our work on the *Cotesia–Pieris* system indicates that behavioural differentiation may occur through the loss or reduced expression of behaviour patterns that were adaptive in the old environment, but turn out to be unprofitable in the new environment. The loss of certain behavioural traits, or their reduced expression, may release the remaining behaviour patterns to be expressed at a higher frequency, leading to a new configuration of behavioural decisions that can be recognized as an adaptation. Such 'gain through loss' may be a likely evolutionary pattern at relatively short time-scales, opening up the way for evolutionary innovations that would have been inaccessible under the original configuration of traits.

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