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Adaptations of an insect to a novel host plant: a phylogenetic approach

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Summary

1. The importance of behavioural *vs* physiological adaptations in the evolution of host associations by herbivorous insects is largely unknown.

2. We compared sister species of beetles, one of which, *Ophraella slobodkini*, feeds on the lineage's ancestral host, *Ambrosia artemisiifolia*, while *O. notulata* has shifted to a novel host, *Iva frutescens*. Assuming *O. slobodkini* represents the features of the *Ambrosia*-feeding ancestor, we asked if behavioural and physiological barriers to utilizing *Iva* existed and if adaptation to these barriers occurred. We also tested for trade-offs between use of novel and ancestral hosts by *O. notulata*.

3. We found evidence that the ancestor of *O. notulata* would have been deterred from feeding on *Iva* and suffered lower conversion efficiency.

4. *Ophraella notulata* appears to have adapted behaviourally by increasing consumption of *Iva*, but we did not detect a significant increase in its physiological capacity to use *Iva*. Additionally, the switch to *Iva* by *O. notulata* did not reduce its physiological capacity to use the ancestral host, *Ambrosia*.

5. Our results suggest that novel host associations may arise from behavioural adaptations, with physiological adaptations a secondary result of behavioural changes. We discuss implications for hypotheses of host shifts and the evolution of specialization.

Key-words: Consumption, conversion efficiency, host shift, phylogeny, plant–herbivore interactions

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Introduction

When sister species of herbivorous insects specialize on different host plants, it may be parsimoniously presumed that their common ancestor fed on one or the other of the two plants (Farrell and Mitter 1990; Funk *et al*. 1995a; Lopez-Vaamonde, Godfray and Cook 2003). One of the species, then, presumably switched to a new host, and abandoned the ancestral host. The proximate cause of a specialized host association is the insect's behaviour: the selection of certain species over others as food and oviposition substrate (Bernays and Chapman 1994). Most insects also have phenological, morphological and other adaptations to their host plant, including physiological adaptations to the plant's toxic or otherwise harmful compounds (Rosenthal and Berenbaum 1992).

Several questions, with broad implications, may be posed about an evolutionary shift from one plant to another. Are there multiple barriers, arising from effects on preference and performance, present for adaptation to a new host? Does the host-shifting species adapt fully to the new host? Is the new, specialized preference (rather than an expanded host range) evolutionarily favoured because of trade-offs in fitness on different plants, with adaptations to the new host reducing fitness on the original host (Futuyma and Moreno 1988; Jaenike 1990; Fry 1996; Thompson 1996)?

The barriers to use of a novel plant include plant features that affect feeding and oviposition preference, especially chemical deterrents and the absence of critical chemical stimulants (Jermy 1984; Bernays and Chapman 1994). Often, postingestive barriers exist as well, because many plant compounds reduce growth and survival (Scriber 1984; Li, Schuler and Berenbaum 2003; Sharma and Norris 1991). Simple measures of growth or survival, sometimes reported as indicators of 'performance' of an insect species on a plant, are affected by both the amount of plant tissue ingested (presumably the effect of 'preference') and the postingestive processing ('performance' *sensu stricto*). Separating these components is necessary in order to judge whether the insect confronts both behavioural and postingestive 'physiological' barriers.

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Differences between populations or species of insects in preference for and performance on a plant appear usually to be under separate genetic control (e.g. Wasserman and Futuyma 1981; Thompson 1988; Fox 1993; Forister 2005), although positive genetic correlations between preference and performance have been reported (e.g. Via 1986; Nielsen 1997; Hawthorne and Via 2001). In at least some cases, the same plant secondary compounds are responsible for both effects (Lindroth, Scriber and Hsia 1988; Hoffmann-Campo, Harborne and McCaffery 2001). These studies suggest that plant features constituting behavioural (preingestive) and physiological (postingestive) barriers may or may not be distinct, but leave open the question of whether or not distinct adaptive changes contribute to an insect's host shift or host range expansion.

Several authors have proposed that behavioural barriers are the most common proximal constraint on diet breadth of phytophagous insects (Futuyma 1983; Jermy 1984; Bernays and Chapman 1994). In some cases they may also be the most important effective barriers, because host-specialized insects sometimes possess the potential for a greater diet breadth physiologically than is realized behaviourally (Karowe 1990; Ballabeni and Rahier 2000; Ueno *et al*. 2001). Consequently, it is not certain that host shifts typically require adaptation to postingestive physiological effects of the new host plant, or that such effects have usually imposed selection for specialized host preference through the presence of trade-offs in performance.

We address these questions by taking a phylogenetic approach to host shifts, and comparing sister species of leaf beetles that we take to represent plesiomorphic, or ancestral (*Ophraella slobodkini*), and apomorphic, or derived (*O. notulata*), host associations. We assume that their common ancestor was about as well adapted to the plesiomorphic host as the species (*O. slobodkini*) that uses it today. By comparing insect species with ancestral and derived host associations, we infer the selective pressures associated with the apomorphic host, the direction and nature of evolutionary changes in responses to these pressures, and the trade-offs associated with this novel host association.

THE SPECIES STUDIED

Species in the leaf beetle genus *Ophraella* (Coleoptera: Chrysomelidae: Galerucinae) are monophagous or oligophagous on various genera of Asteraceae (LeSage 1986; Futuyma 1990). *Ophraella slobodkini* Futuyma (Futuyma 1991) is distributed in peninsular Florida, west along the Gulf Coast to Louisiana; its sole recorded host plant is common ragweed, *Ambrosia artemisiifolia* (Heliantheae: Ambrosiinae), which occurs in upland habitats. Its sibling (i.e. morphologically similar) species, *Ophraella notulata* (Fabricius), is almost entirely restricted to salt marshes along the Atlantic and Gulf coasts of the United States, where it feeds on marsh elder, *Iva frutescens*, a species found only in salt marshes and a member of the same subtribe (Ambrosiinae) as *Ambrosia*. This species has also been taken from *Iva annua* in a Louisiana locality (Futuyma 1991). Specimens from inland localities have been attributed to *O. notulata* (LeSage 1986), but their identity is uncertain, because these sibling species are morphologically almost indistinguishable. The host plants are not syntopic, since they occupy different habitats, but they sometimes occur in close proximity at the ecotone between upland and salt marsh. Nevertheless, their ranges are largely allopatric, as are the ranges of their associated *Ophraella* species. Based on mitochondrial gene sequences of 12 species in the genus *Ophraella*, Funk *et al*. (1995a,b) concluded that *O. slobodkini* and *O. notulata* are sister species (i.e. each other's closest relatives) that diverged from a common ancestor perhaps as long ago as 5·7 Mya. Data from multiple geographical populations indicate that each species forms a monophyletic clade. The distribution of host–plant associations on the mtDNA phylogeny implies that *Ambrosia artemisiifolia* is probably the ancestral host, relative to *Iva frutescens*.

Methods

HOST PLANT MATERIAL

Seeds of *Ambrosia artemisiifolia* were collected on and near the campus of the State University of New York (Stony Brook, NY) and started *en masse* in flats ($52 \times 25 \times 6$ cm^3) containing Pro-Mix BX (Premier Horticultural Ltd, Dorval, Canada) using a growth chamber with constant light. After germinating (3 days), seedlings were individually transplanted to pots (1·8 l) with Pro-Mix BX and placed in a greenhouse with supplemental fluorescent lighting (16:8 h light : dark). Two weeks after transplanting, seedlings were fertilized with Miracle-Gro Professional Excel Cal Mag 15:5:15 (N:P:K) (The Scotts Company, Marysville, OH) at a concentration of 300 ppm nitrogen. Foliage from *Iva frutescens* was collected from an established native population in the salt marsh at Flax Pond Marine Research Laboratory (Setauket, New York). Pennings, Siska and Bertness (2001) reported that northern populations of *Iva frutescens* are more 'palatable' to *O. notulata* (i.e. preferred in choice tests) than southern populations; our use, perforce, of northern plant material does not take into account possible effects of this difference.

HERBIVORES

Ophraella notulata were collected from *Iva* at Flax Pond Marine Research Laboratory. Adults were placed in clear plastic cylindrical tubes with cuttings of *Iva*. *O. slobodkini* were collected in Gainesville, Florida, and maintained in the laboratory on *Ambrosia* grown as previously described. Over 1 week, approximately 325 eggs per species were collected and placed on moistened filter paper (VWR Qualitative #413, VWR International, West Chester, PA) in Petri dishes. Eggs were checked daily, and newly hatched larvae were placed individually in 5·5 cm diameter Petri dishes lined with moistened filter paper and containing foliage of their native host, with fresh leaf material provided every two days. Larvae were checked daily for moulting and started in an experimental treatment the day they moulted to the final (third) instar.

FEEDING ASSAYS

Because of the small size of *Ophraella* larvae (roughly 2 mg at the start of the final instar), assays were conducted in cages of weighing paper (VWR Brand, VWR International) that were housed within the cap of a 1·5-ml microfuge tube (Marsh Biomedical Products, Rochester, NY). Into a cap lined with a weighing paper disk, a larva was placed along with leaf disks from a host plant. A second weighing paper disk was set across the cap of the microfuge tube and the tube was carefully pressed onto the cap, sealing the larva and host plant material inside. Each paper disk had nine pin holes at the centre to allow for ventilation; additionally, several holes were made in the top and side of the microfuge tube. To prevent desiccation of leaf material, the bottom of each microfuge tube was removed and the open end of the tube placed in water in a 24-well plate (BD Falcon, Lincoln Park, NJ). The tubes were covered with damp paper towels and placed inside a sealed plastic box.

Prior to the start of an assay, larvae along with weighing paper disks were weighed to 0·001 mg on a Cahn C-32 microbalance (Cahn Instruments, Cerritos, CA). Each day, one-quarter of the larvae were randomly selected and dried to a constant mass at 60 °C to estimate the correlation between wet and dry mass; the rest were assigned to an experimental treatment. Larvae were given leaf discs (diameter = 0·5 cm) of either *Iva* or *Ambrosia*, in a fully crossed two-factorial design, consisting of host plant (*Iva vs Ambrosia*) and herbivore species (*O. slobodkini vs O. notulata*). Paired leaf discs were taken on opposite sides of the principal veins of a leaf, with one disc fed to a larva while the other was dried to provide an estimate of initial dry mass of the eaten disc.

Assays lasted 3 days and fresh leaf disks were provided daily, with larvae fed *ad libidum* during the assay. Data on host utilization and growth were analysed only for larvae that survived to the end of the 3-day trial. Final sample sizes were: *O. slobodkini* on *Ambrosia* = 19, *O. slobodkini* on *Iva* = 18, *O. notulata* on *Ambrosia* = 29, and *O. notulata* on *Iva* = 29.

At the end of a trial, larvae, uneaten leaf tissue, and weighing papers with frass were dried and weighed as previously described. Mass of leaf tissue eaten was the difference between eaten and uneaten discs for each leaf disc pair. Mass of frass was the difference in the initial and final mass of weighing paper cages. Biomass gained by larvae was the difference between final dry mass and initial dry mass, estimated using a regression of initial dry mass on initial wet mass for a randomly selected subset of larvae.

DATA ANALYSIS

Data were analysed following a two-factorial design of host plant species, herbivore species and their interaction. We tested for effects on consumption, dry mass gained by larvae (relative growth rate (RGR)), approximate digestibility of host plant (AD), efficiency of conversion of ingested plant material (ECI), and efficiency of conversion of digested plant material (ECD) (Waldbauer 1968; Raubenheimer and Simpson 1992).

Following Raubenheimer and Simpson (1992), all of these analyses were based on an analysis of covariance (PROC GLM in SAS 8·0e) (SAS Institute 1999). ANCOVAS for consumption and RGR used the covariate of initial wet mass. AD was analysed as an ANCOVA of leaf tissue digested (mass of leaf tissue consumed − mass of frass egested) with the covariate of mass of leaf tissue consumed (both measured as mg dry mass). All means were calculated as least-squares means (LSMEANS option in SAS).

ECI and ECD were initially analysed with ANCOVAS of dry mass gained by larvae with the covariates of leaf tissue consumed and leaf tissue digested, respectively. For ECI and ECD, the slopes of dry mass gained onto the covariate were not homogeneous, preventing further use of ANCOVA. We therefore tested for effects on conversion efficiency by using a general linear model (PROC GLM) that contained the factors of host plant and herbivore species, and the continuous variable of leaf tissue ingested or digested for ECI and ECD, respectively. In both analyses, regression intercepts did not differ among regression lines, as indicated by the lack of significant effects for host plant, herbivore species or their interaction (see lines 1–3 in Table 3). Consequently, tests for effects of host plant, herbivore species and their interaction on conversion efficiency were conducted by comparing the steepness of the regression slopes for gain in biomass as a function of consumption (see lines 5–7 in Table 3). The comparison of regression slopes has been applied in other studies to analyse growth as a function of consumption for phytophagous insects across diets (e.g. Miller and Feeny 1983; Usher and Feeny 1983). Steeper regression slopes indicate greater conversion efficiency and are tested statistically as the interaction of consumption with the other factors in the model (e.g. Herbivore ∗ Consumption). Pairwise comparisons were made among regression slopes following the formulae given in Sokal and Rohlf (1995), with the significance level set at 0·009 based on the Dunn-Šidák correction assuming six pairwise comparisons (Sokal and Rohlf 1995).

Because leaf mass eaten was estimated from paired leaf discs it had an associated measurement error that is expected to bias regression slopes downward (Snedecor and Cochran 1989). To address this issue, we quantified the measurement error between leaf punches, and then used a corrected least-squares regression (Snedecor and Cochran 1989) to calculate the expected regression slope and associated standard error in the absence of this measurement error. Corrected slopes were tested to confirm differences among regression slopes detected using the general linear model. All pairwise comparisons were based on corrected slopes and their adjusted standard error. Additionally, to assess the potential effects of measurement error on the ANCOVA-based test of approximate digestibility (AD), we also analysed AD with a ratio-based metric ((consumption – frass)/consumption) following Waldbauer (1968).

Results

There was a significant interaction between host plant and herbivore species for the response variables of consumption and relative growth rate (Table 1). Both herbivores consumed similar amounts of leaf tissue on their native hosts. Each consumed more of its native host than of the congener's host, but this effect was greater for *O. slobodkini* (Fig. 1a). If *O. slobodkini* is representative of an *Ambrosia*-feeding common ancestor, this pattern implies that the switch from *Ambrosia* to *Iva* by the *O. notulata* lineage resulted in lower rates of consumption than occurred on the ancestral host, but that *O. notulata* eventually overcame this deterrent effect. Additionally, the host shift of *O. notulata* onto *Iva* was accompanied by reduced consumption of the ancestral host, *Ambrosia*.

Ophraella slobodkini gained significantly more biomass on *Ambrosia* than was observed in the three other combinations of herbivore by host plant, but displayed the smallest gain in biomass on *Iva*. The gain in larval biomass for *O. notulata* was similar and statistically equivalent on the two host plants, and intermediate to the biomass mass gain by *O. slobodkini* on these hosts (Fig. 1b). This result implies that the initial switch to *Iva* by the *Ambrosia*-feeding ancestor of *O. notulata* would have resulted in lower growth rate, and that *O. notulata* has adapted to *Iva*, as it now displays greater biomass gain on this host than *O. slobodkini.* The slower growth rate of *O. notulata* than *O. slobodkini* on *Ambrosia* indicates that, for *O. notulata*, adapting to *Iva* was accompanied by diminished growth on *Ambrosia*.

Both analyses of approximate digestibility (AD) showed that it varied significantly with host plant and herbivore species (Table 2). For both herbivores, AD

Table 1. Analysis of covariance for rate of feeding (Consumption) and relative growth rate (RGR) of *Ophraella* spp. Factors in the model included host plant (*Iva vs Ambrosia*), herbivore species (*O. slobodkini vs O. notulata*), and the initial wet mass of larvae as the covariate

	Consumption		RGR	
Source	df	*F*	df	*F*
Host plant	1	9·27**	1	47·81***
Herbivore	1	8·81**	1	1·31
Herbivore * Host plant	1	95·79***	1	100·21***
Initial mass (covariate)	1	6·86*	1	1·26
Error	90		90	

*P < 0·05; **P < 0·01; ***P < 0·001.

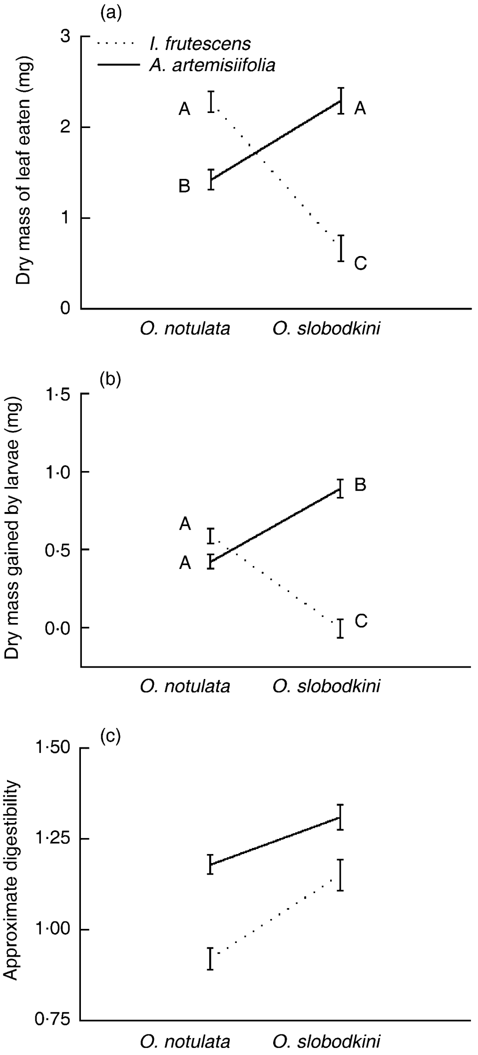

Fig. 1. (a) Larval consumption, (b) relative growth rate and (c) approximate digestibility of leaf tissue. Both consumption and growth were corrected for initial wet mass of larvae. Approximate digestibility is the mass of leaf tissue digested (consumption – frass) corrected for the total amount of leaf tissue consumed. The ends of each line represent a sample mean, calculated as a least-squares mean, and error bars are the standard error of the mean. In (a) and (b), letters indicate significant pairwise differences.

Table 2. Analysis of covariance and analysis of ratios for approximate digestibility (AD) of leaf tissue. In the ANCOVA, leaf tissue digested is the response variable (leaf tissue consumed (mg) – frass (mg)), and leaf tissue consumed is the covariate. The ratio of approximate digestibility was calculated as ((leaf tissue consumed – frass)/leaf tissue consumed). Factors in the model included host plant (*Iva vs Ambrosia*) and herbivore species (*O. slobodkini vs O. notulata*)

	AD ANCOVA		AD ratio	
Source	df	*F*	df	*F*
Host plant	1	45·74**	1	18·75***
Herbivore	1	33·39***	1	8·47**
Herbivore * Host plant	1	1·42	1	0·17
Leaf tissue eaten (covariate)	1	764·58***	–	–
Error	90		91	

*P < 0·05; **P < 0·01; ***P < 0·001.

Table 3. General linear model for conversion efficiency of ingested (ECI) and digested (ECD) leaf tissue. The response variable is dry mass gained by larvae. Factors in the model included host plant (*Iva vs Ambrosia*), herbivore species (*O. slobodkini vs O. notulata*), and the continuous variable of consumption. In the analysis of ECI, consumption represents the mass of leaf tissue consumed, and for ECD, consumption represents the mass of leaf tissue digested (leaf tissue consumed (mg) – frass (mg)). Lines 1–3 (Host plant, Herbivore and Herbivore ∗ Host plant) test for differences in intercept; line 4 tests the significance of the regression of growth on consumption; lines 5–7 test for differences in regression slope

		F	
Source	df	ECI	ECD
Host plant	1	0·14	2·38
Herbivore	1	0·18	1·94
Herbivore ∗ Host plant	1	0·65	0·29
Consumption	1	228·56∗∗∗	195·47∗∗∗
Consumption ∗ Host plant	1	16·17∗∗∗	16·04∗∗∗
Consumption ∗ Herbivore	1	1·01	0·72
Consumption ∗ Host plant ∗ Herbivore	1	1·96	5·08∗
Error	87		

$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

was greater on *Ambrosia* than on *Iva*. For both host plants, AD was higher for *O. slobodkini* than *O. notulata* (Fig. 1c).

The efficiency of conversion of ingested food (ECI) differed significantly between host plants, as indicated by an interaction between leaf tissue ingested and host plant (Table 3). This effect was present when data were reanalysed with a corrected least-squares regression ($F = 20{\cdot}43$; df = 1, 91; $P < 0{\cdot}0001$). This significant interaction arose because, for both species of herbivores, regression slopes for gain in larval biomass per unit leaf tissue consumed were lower on *Iva* than on *Ambrosia* (Fig. 2a), indicating that a smaller proportion of consumed *Iva* than *Ambrosia* was converted to larval biomass. In contrast, the conversion efficiency of digested food (ECD) differed among the four combinations of host plant and herbivore species, as indicated by a significant three-way interaction among leaf tissue digested, host plant and herbivore species (Table 3). This result was also found when data were reanalysed with a corrected least-squares regression ($F = 6{\cdot}15$; df = 3, 87; $P < 0{\cdot}001$). This interaction means that the regression slope of larval biomass gained per unit of leaf tissue digested differed among the four combinations of herbivore and host plant. Pairwise comparisons of regression slopes indicated that ECD was significantly lower for *O. slobodkini* on *Iva* than on *Ambrosia* (Fig. 2b).

The values of ECI and ECD for *O. slobodkini* suggest that the common ancestor of these *Ophraella* species would have suffered lower conversion efficiency on *Iva* than on *Ambrosia*. However, because *O. notulata* and *O. slobodkini* were found not to differ in ECI or ECD on *Iva*, there appears to have been little, if any, increase in the physiological capacity of *O. notulata* to use its current host. Although *O. notulata* gained more mass than *O. slobodkini* when reared on *Iva* (Fig. 1b), this difference may be explained entirely by its higher consumption (Fig. 1a). Furthermore, the lack of any significant differences in ECD and ECI between *O. notulata* and *O. slobodkini* on *Ambrosia* suggests that the acquisition of *Iva* by *O. notulata* has not decreased its conversion efficiency of *Ambrosia*.

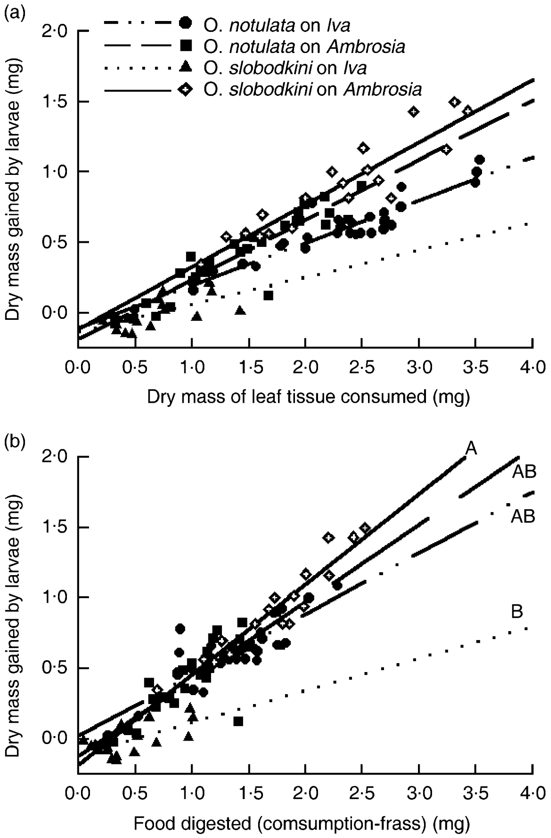

Fig. 2. Conversion efficiency of (a) ingested and (b) digested leaf tissue. Symbols represent measurements for individual larvae and lines are the linear regressions for the several treatments (e.g. *Ophraella notulata* fed *Ambrosia artemisiifolia*). Steeper regression slopes demonstrate that a higher proportion of leaf material was converted to larval biomass. For Fig. 2(b), letters indicate significant pairwise differences among regression slopes.

Difference in consumption may bias measures of conversion efficiency, with lowered rates of consumption generating decreased levels of conversion efficiency (Usher and Feeny 1983; Horton and Redak 1993). This should not affect results for ECI because consumption was similar between *Iva* (0·211–3·536 mg) and *Ambrosia* (0·318–3·432 mg). For ECD, consumption of *Iva* by *O. slobodkini* was lower than the other experimental combinations, and ECD may have been underestimated. The absence of this bias should increase the steepness of the regression slope, such that the pattern for ECD more closely resembles ECI. ECD would nevertheless display the same significant effect of host plant as ECI (Table 3), consistent with the proposition that switching to *Iva* lowered conversion efficiency in the ancestor of *O. notulata* and that there has been little adaptation to *Iva* by *O. notulata.*

Discussion

These sister species differ greatly in behavioural response to the host plants: each consumed more of its own than of the congener's host, a difference that is particularly pronounced in *O. slobodkini* (Fig. 1a). Likewise, each insect species gained more mass feeding on its own host than the host of its congener (Fig. 1b). The differences in mass gain appear to be explained mostly by differences in consumption. Measures of mass gain corrected for consumption (ECI, ECD) indicate significantly lower conversion efficiency of *Iva* than *Ambrosia* by *O. slobodkini*, but not significantly greater conversion efficiency for *O. notulata* than *O. slobodkini* on *Iva* (Table 3, Fig. 2). Calculations of approximate digestibility (AD) indicate that for both insects, *Iva* is a less digestible plant than *Ambrosia* (Table 2, Fig. 1c). Thus, our data provide evidence for the evolution of behavioural adaptation to the host plants, but no demonstrable evidence of adaptation in postingestive physiological characters. While larger sample sizes might have enabled us to detect a significant difference in postingestive characters between *O. notulata* and *O. slobodkini* on *Iva*, it nevertheless appears that behavioural adaptations are far more pronounced than physiological adaptations. Keese (1998) showed that larval consumption, survival and growth of *O. slobodkini* were much reduced on *Iva* compared with *O. notulata* on *Ambrosia*. Our data suggest that differences in consumption may account in large part for the differences in performance that Keese (1998) described.

On the basis of phylogenetic considerations described above, we hypothesize that the common ancestor of these species of *Ophraella* fed on *Ambrosia artemisiifolia*, and that *O. notulata* represents a shift to a new host plant, *Iva frutescens*. If we assume that *O. slobodkini* represents the ancestral state, then we may conclude that when the lineage first colonized *Iva*, it faced both a substantial behavioural barrier to larval feeding and a moderate postingestive barrier to efficient digestion. Since then, *O. notulata* has clearly adapted in behaviour, but appears to have undergone little physiological adaptation. Keese (1996) found that the expression of preference for these plants by F1 hybrid larvae differed from the pattern of larval survival, suggesting that these traits are inherited at least partly independently. Behavioural and physiological adaptations of *O. notulata* to *Iva* therefore are probably based on different traits, as has been found from many other studies of phytophagous insects (Futuyma and Peterson 1985, Schoonhoven, Jermy and van Loon 1998). Moreover, *O. notulata* provides another example of discordance between behavioural and physiological adaptation of insects to their host plants (Orians *et al*. 1997; Kagata and Ohgushi 2001; Forister 2004), consistent with the hypothesis that host shifts are initiated by evolution of behaviour (Dethier 1970; Futuyma 1983; Futuyma, Keese and Scheffer 1993). Behavioural shifts in ecological niche often precede, and consequently impose selection on, the evolution of morphological and physiological characters (Mayr 1963, p. 604; Wcislo 1989; Odling-Smee, Laland and Feldman 2003).

If *Iva* was and still is nutritionally inferior to *Ambrosia*, why did the *O. notulata* lineage shift to and become specialized on this plant? Some factor other than host-plant quality was presumably the driving force behind this host shift. Interspecific interactions such as competition (Denno, McClure and Ott 1995) or predation (Jeffries and Lawton 1984; Berdegue *et al*. 1996) may have favoured a shift. Keese (1997) found a lower level of predation on *Ophraella* eggs on *Iva* than on *Ambrosia*, but this advantage was countered by a higher rate of larval parasitism on *Iva*; Keese (1997) therefore considered it unlikely that the advantage of shifting to *Iva* lay in escaping predation. Alternatively, adaptation to a novel plant, even if inferior, may be favoured simply by its availability, if the normal host is locally rare (Strong, Lawton and Southwood 1984), which could occur at the transition between the upland habitat of *Ambrosia artemisiifolia* and the salt-marsh habitat of *Iva frutescens*.

Loss of adaptation (e.g. feeding response) to the ancestral host plant might occur because of a trade-off in fitness on the two hosts (Ehrlich and Raven 1964; Futuyma and Philippi 1987; Fry 1996) or because spatial isolation on the new host allows fixation of disabling mutations in genes that are expressed only when occupying the ancestral host (Kawecki, Barton and Fry 1997; see also Holt and Gaines 1992; Holt and Gomulkiewicz 1997). We found little evidence that *O. notulata* suffers a physiological trade-off as a result of switching to *Iva*. Although *O. notulata* gained less biomass on *Ambrosia* than did *O. slobodkini*, this appeared to result primarily from a reduction in consumption (Fig. 1a), with these species displaying similar levels of conversion efficiency on *Ambrosia* (Fig. 2). Likewise, Keese (1998) reported that indicators of larval performance on *Ambrosia* of *O. notulata vs O. slobodkini* differed marginally or not at all. We cannot rule out possible trade-offs in features other than postingestive physiology, such as host-finding and recognition (Bernays 2001) or adaptations to the habitats in which the host plants occur.

A residual capacity to use ancestral hosts has been described for other insects (Nitao *et al*. 1991; Ikonen *et al*. 2003). If this pattern is common, evolutionary reversals to an ancestral host association may be more likely than a shift to a novel host plant, especially if, as in this case, the novel plant presents multiple barriers. Although cases of trade-offs in physiological adaptation to different host plants have been described for some insect species, our study joins a long list of cases in which trade-offs of this kind have not been detected (summaries in Jaenike 1990; Keese 1998; but see Mackenzie 1996). Such trade-offs have often been proposed to underlie speciation and the evolution of specialization in phytophagous insects, but other factors may often be more likely, including neural constraints affecting host recognition (Bernays 2001) or, possibly, physiological trade-offs in adaptation to abiotic conditions.

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