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Macro-evolutionary trade-offs in the tephritid genus *Urophora*: benefits and costs of an improved plant gall

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ABSTRACT

The species of the tephritid genus *Urophora* differ greatly in the structure and function of the galls, which they induce on their Cardueae host plants. As this suggests the existence of trade-offs, we studied the benefits and costs of *Urophora* galls of different complexity using *U. quadrifasciata*, an oligophagous species with a primitive achene gall, and *U. jaceana*, a monophagous species with a complex multilocular ovariole-receptacle gall. Both *Urophora* species share *Centaurea jacea* as host plant and may co-exist in the same host populations without any sign of interspecific competition. Therefore, *C. jacea* was used in our tests as the standard host plant. We examined life-history data of the two species and tested the hypothesis that they respond in different ways to differences in host quality. Oviposition behaviour and larval development were studied on unfertilized and well-fertilized test plants grown under standardized nutrient regimes. The females of the specialist tephritid species, *U. jaceana*, had a much higher egg load, higher oviposition decisiveness, reduced time costs per deposited egg and they were able to select and adjust clutch size to the quality of the host plant. This oviposition behaviour maximized reproductive fitness, as even high larval densities in galls on well-fertilized hosts had no negative effect on larval weight. In addition, larvae in well-fertilized buds developed more biomass and produced females with a higher egg potential. Females of the generalist species, *U. quadrifasciata*, did not respond to differences in host quality. They could use flower heads during a broader temporal window, but had a lower reproductive reward per handling time and the larvae extracted much less energy and nutrients from the host. However, their development was distinctly faster, making a second generation per annum possible. Moreover, *U. quadrifasciata* has a much broader host range and is better adapted for long-distance dispersal, which has made it a very successful pioneer species in North America. The comparison of the two *Urophora* species shows that the evolution of a refined oviposition behaviour and the formation of a complex gall, which allows the import of nutrients via a newly formed vascular system, greatly increases the energy and nutrient rewards, as long as stable host populations can be exploited. On the other hand, the advanced gall type of *Urophora* involves higher costs of developmental time, univoltine generations and a high dependence on a specific host plant.

Keywords: effect of nitrogen, evolution of plant galls, host plant quality, host range, interspecific competition, oviposition behaviour, Tephritidae, *Urophora*.

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INTRODUCTION

Macroevolutionary trade-offs (Stearns, 1992) are independent phylogenetic events in which a change in one trait that decreases fitness is linked to a change in another trait that increases fitness. In phytophagous insects, such trade-offs can be expected where monophagous specialists co-exist with generalist congeners, as it is postulated that phytophagous specialists can make up for the disadvantage of being confined to a single host. The means by which this compensation can be achieved are manifold and are of general interest for the study of host specialization in phytophagous insects (Bernays and Graham, 1988; Futuyma and Moreno, 1988; Jaenike, 1990; Farrell *et al.*, 1992; Bernays and Chapman, 1994; Bernays, 1998). In this paper, we examine trade-offs connected with the evolution of plant galls.

The induction of plant galls, specific and often very intricate plant structures that provide food and shelter (Ananthakrishnan, 1984), is the evolutionarily most advanced way for phytophagous insects to exploit their resources. The ability to induce complex plant galls is an evolutionarily derived (apomorphic) character state if compared to phytophagous insect taxa with or without primitive galls (Steck, 1981; Freidberg, 1984). The evolution of complex galls may imply costs, as the range of host plants (Meyer, 1987) and the window for oviposition suitability (Headrick and Goeden, 1998) become narrower and galls may form recognizable targets for specialized parasitoids (Hawkins, 1994). Which trade-offs compensate for such disadvantages faced by specialized gall makers?

The tephritid genus *Urophora*, which comprises specialized gall-formers as well as a generalist species, may help us to answer this question. The members of this taxon are almost exclusively associated with the flower heads of Cardueae host plants and vary considerably in the way in which they exploit their hosts. Some species inhabit flower heads without producing galls (Merz, 1994), another makes a very primitive gall (*U. quadrifasciata*), but most form galls of differing complexity (Arnold-Rinehart, 1989). The species also differ in the breadth of their host ranges. A particularly interesting pair of species is *U. quadrifasciata*, which accepts almost all *Centaurea* spp. as hosts (Zwölfer, 1965), and the monophagous *U. jaceana*, which in our study area breeds only in the heads of the brown knapweed (*Centaurea jacea*). Both *Urophora* species may co-exist in the same host-plant population. We benefit from the fact that there are several publications on the ecology of *U. quadrifasciata* (Harris, 1980a,b) and *U. jaceana* (Wadsworth, 1914; Varley, 1947; Dempster *et al.*, 1995b). Berube (1980) and Myers and Harris (1980) investigated the competitive co-existence of *U. quadrifasciata* and another gall-forming *Urophora* species, *U. affinis*, in Canada, where both species have been introduced as biological control agents. These two studies have provided interesting information on the resource utilization and dispersal behaviour of *U. quadrifasciata*. Arnold-Rinehart (1989) and Canadian studies summarized by Harris and Shorthouse (1996) have analysed the different ways in which the larvae of *U. quadrifasciata*, *U. jaceana* and other *Urophora* species with specialized galls gain their nutrients in Cardueae flower heads.

In this study, we compared the monophagous and highly specialized *U. jaceana* with its oligophagous and much less specialized congener, *U. quadrifasciata*, in a series of experiments in which the two species were confronted with a common host plant (*Centaurea jacea*) grown from seeds under two different nutrient regimes. We analysed the behaviour of adults, larval performance and its fitness consequences and, in addition, we evaluated

the life-history traits of the two species. Our aim was to test the hypothesis that *U. jaceana* makes up for its restricted resource base by an increased ability of the females to respond to differences in host quality and by a more efficient use of the host resources by the larvae.

BIOLOGICAL AND ECOLOGICAL DATA

Distribution, host range and dispersal capacity

Urophora jaceana occurs in western, central and parts of eastern Europe (England, Belgium, France, Germany, Hungary, Poland, Ukraine, western Russia) (Leclercq, 1967; Foote, 1984; Korneev and White, 1996) and as an accidental introduction in a small part of eastern Canada (Newfoundland, Nova Scotia) (Shewell, 1961). Its host range is restricted to a few closely related *Centaurea* spp. in the subgenus *Jacea*: *C. jacea*, *C. nigra*, *C. pratensis*, possibly also *C. pseudophrygia* (Zwölfer, 1965; Merz, 1994). *Urophora jaceana* is monophagous on *C. jacea* in our study area.

The polytypic species *U. quadrifasciata* has been recorded in Europe, North Africa and parts of Asia (Turkey, Israel, Iran, Transcaucasia, Kazakhstan) (Foote, 1984). It was accidentally introduced to, and has become established in, Australia (Merz, 1994). White and Korneev (1989) have split *U. quadrifasciata* into three morphologically different subspecies, two of which are associated with the *Centaurea* subgenus *Calcitrapa*. The subspecies *U. quadrifasciata quadrifasciata* has been reared from the flower heads of 18 *Centaurea* spp. belonging to the subgenera *Centaurea*, *Acrolophus*, *Jacea* and *Cyanus* and from heads of *Serratula tinctoria* (Zwölfer, 1965, and unpublished personal data; Merz, 1994). A single personal rearing record of an unknown subspecies of *U. quadrifasciata* has been obtained from *Microlonchus salmanticus*. The subspecies *U. q. quadrifasciata* was introduced to western Canada in 1972 in connection with biological control projects against the introduced weed species *Centaurea diffusa* and *C. maculosa*. From there it spread naturally to the USA. By redistribution and by its own dispersal capacity, it has now become established in 16 states in the USA. In North America, it attacks not only the two target host plants but has spread to *Centaurea cyanus*, *C. jacea x nigra*, *C. solstitialis* and *C. virgata* (Julien and Griffiths, 1999).

Follow-up studies of *U. quadrifasciata* in Canada and the USA have demonstrated that this species has a considerable capacity for dispersal over long distances. According to Harris (1986), it has dispersed over at least 400 km in the 9 years (i.e. 18 generations) since its release in British Columbia. Its rapid spread in North America, where it now occupies a large part of Canada and the USA, is in distinct contrast with *U. jaceana*, which, according to museum specimens, must have been introduced to Canada before 1923. Nevertheless, it still inhabits only a small area despite its host plant, *Centaurea nigra*, being widely spread in Canada and also being reported from the northeastern USA (Shewell, 1961). In release experiments with marked *U. jaceana*, Dempster *et al.* (1995b) found that during one generation the maximal distance covered was 1.2 km, a dispersal range that corresponds well with observations made on other tephritid species associated with Cardueae hosts. This suggests that the capacity of long-distance dispersal of *U. quadrifasciata* is about 20 times greater than that of *U. jaceana*.

Oviposition, gall formation and gall structure

Females of both species insert their ovipositor between the bracts of the closed flower buds and examine the interior structure of the bud. If oviposition takes place, eggs are laid among the developing stamens. Eggs of *U. quadrifasciata* are often introduced into the individual florets of the capitulum. Females of *U. jaceana* prefer to oviposit into an earlier bud stage (Table 1) than females of *U. quadrifasciata*. The larva of *U. jaceana* hatches as a second instar from the egg, whereas that of *U. quadrifasciata* emerges as a first larval instar (White and Clement, 1987).

The *U. quadrifasciata* larva stays within and mines a fertilized ovariole, stimulating the fascicular parenchyma and the young undifferentiated tissues to proliferate. Small patches of nutritive tissue differentiate at the walls of the larval cavity, but the main food resource is the fascicular parenchyma of the vascular bundles. In contrast to all other *Urophora* species, the galls of *U. quadrifasciata* are not lignified and pupation occurs within the inflated remainders of the achene. On average, the growth and maturation of the gall is complete after 20 days (Varley, 1947; Arnold-Rinehart, 1989; Harris and Shorthouse, 1996).

A detailed description of the morphogenesis and structure of the ovariole-receptacle gall of *U. jaceana* is given by Arnold-Rinehart (1989). As the larva tunnels through the ovariole, it stimulates the lower part to proliferate and causes the upper part of the receptacle to grow together with the ovariole. Gall parenchyma arises mostly out of the receptacle tissue and the procambial strands, which form in the newly built parenchyma. Primary nutritive tissue develops in small patches around the larval cavity. During this growth phase, the second-instar larva of *U. jaceana* consumes only little nutrient. In the maturation phase of the gall, secondary nutritive cells develop out of the procambial strands. They are the main food resource for the now rapidly growing larva. The tissue in the periphery of the gall lignifies more and more and the growth of the gall is complete after about 30 days. *Urophora jaceana*

Table 1. Life-history data of *U. jaceana* and *U. quadrifasciata*

	U. jaceana	*U. quadrifasciata*	*P*(*t*-test)
Generations per annum	1	2	
Fresh weight of females (mg)	3.34–5.71	1.65–2.13	
Egg load (mean)			
age 4 days	144.1 ($n = 86$)	48.7 ($n = 108$)	< 0.0001
age 6 days	175.5 ($n = 35$)	55.0 ($n = 30$)	< 0.0001
age 8 days	170.8 ($n = 36$)	65.6 ($n = 38$)	< 0.0001
Diameter of accepted *C. jacea* buds (range and mean)	2.4–7.2 (4.7) mm	5.5–10.1 (8.0) mm	
Bud acceptable over:	15 days	20 days	
Duration of gall development	about 30 days	about 20 days	
Time required for emergence of adults after hibernation* (median, interquartile range)	males: 25 (21–29) days, $n = 426$ females: 26 (22–32) days, $n = 419$	males: 17 (15–21) days, $n = 1633$ females: 18 (15–21) days, $n = 1378$	

* Material kept until June/July at 5°C and then transferred to room temperature.

larvae hibernate and pupate within their gall. The main difference between the two gall types is the way in which the larvae gain nutrients and energy. *Urophora quadrifasciata* is largely limited to nutrients supplied from adjacent gall tissue, whereas *U. jaceana* also has nutrients at its disposal that are imported via the newly generated vascular system of the gall (Harris, 1980b; Arnold-Rinehart, 1989; Harris and Shorthouse, 1996).

MATERIALS AND METHODS

Laboratory and field cage experiments

Our experiments were carried out with adults of *U. quadrifasciata* and *U. jaceana*, which were reared from samples of *Centaurea jacea* flower heads collected in the autumn and winter of 1994 and 1995 in the vicinity of Bayreuth (north-eastern Bavaria) and kept during the winter under outdoor conditions. The material was subsequently stored at +5°C and, whenever needed, transferred to room temperature. Adult males and females were kept together on filter paper in Petri dishes (9 cm diameter). They had free access to a diluted honey solution. As host plant in the various tests we used *C. jacea*, which was grown from seeds under two different standardized conditions. The unfertilized group of plants received a mixture of three parts peat to one part sand (pH = 7). To grow the well-fertilized group, a standard fertilizer (Osmocote, 3 $kg \cdot m^{-3}$) was added. The morphological characters of the two plant groups were assessed and the achenes were analysed for Kjeldahl nitrogen content (Strauch, 1965). Differences between the two groups of test plants are summarized in the section on the 'Effects of host quality'. Burkhardt (1999) provides further details on the cultivation and the consequences of the two nutrient regimes.

The host selection behaviour of *U. quadrifasciata* and *U. jaceana* was studied in three series of experiments. The tests were carried out in walk-in cages ($2 \times 2 \times 2$ m) under field conditions (pre-alighting tests), in smaller cages ($0.5 \times 0.5 \times 0.7$ m) placed in the open air (sequential choice tests with whole plants) and in a small arena ($(5.5\text{ cm})^2 \cdot \pi \cdot 15$ cm) in a climatized room (video-recorded choice tests with buds). In the first two types of tests, potted plants of the two treatment groups were selected at random and used if at least two flower buds were in a developmental phase acceptable for oviposition. For the arena experiments, stalks with one flower bud and 5–6 leaves were cut from well-fertilized and unfertilized plants. At the start of all experiments, the flies used had had no prior host contact.

In the pre-alighting tests (9 tests with *U. quadrifasciata* and 8 with *U. jaceana*), one potted host plant of each treatment group was exposed to 30–70 females (at least 4 days old) and the same number of males, which were released at a distance of 1 m. For 3 (*U. quadrifasciata*) and 2 (*U. jaceana*) hours, respectively, the test plants were monitored continuously. Each alighted adult was removed from the experiment.

In the sequential choice test, a single mated female (4 days old) was released at the rosette of a plant of one of the treatment groups and its behaviour was recorded until the female left the plant. The test series was continued if contact with the flower was made. After 2 days, the same female was confronted with a plant of the other treatment group. Five series of tests were conducted with *U. quadrifasciata* and 12 with *U. jaceana*.

In the arena experiments, single mated females (4 days old) were offered a choice between two stalks with flower buds, one of each treatment group. Every test was video-recorded, lasted 60 min and was only counted if probing of one of the buds occurred.

Flower buds used in the sequential and video-recorded choice tests were dissected under a stereomicroscope to count the number of eggs.

The behaviour of the females of *U. quadrifasciata* and *U. jaceana* searching for hosts or being active on the host plant has been described in detail by Burkhardt (1999). On the flower bud, the female may be either 'examining' (moving with the oviscape and head bent downwards) or 'probing' (tip of the oviscape contacting the surface of the bud, the eversible ovipositor sheath and the aculeus introduced into the flower bud). Probing may or may not result in the deposition of eggs, which can only be verified by a subsequent dissection of the flower bud. Other behaviours include 'orientation' (a particular position of head, body and wings), 'walking' (on stalks and leaves), 'flying' (often also used to cover very short distances), 'resting' and 'grooming'. In the arena experiments, there was a discernible orientation of the females towards *C. jacea* flower buds on isolated stalks from 10 cm away. The experiments in walk-in cages suggested that the adults of the two *Urophora* spp. could visually recognize their host plant from a distance of 1 m or more.

Experiments to assess the larval development of *U. jaceana* took place under a day/night regime of 14/10 h, 23/15°C and 70/90% relative humidity. For a detailed description of the methods, the reader is referred to Burkhardt (1999).

Analysis of field data

Data on the host ranges of the two *Urophora* spp. originate from a systematic survey of the insect fauna of Cardueae in western, southern and central Europe, which began in 1961 (Zwölfer, 1965) and continued into the 1990s. It has yielded over 200 personal records for *U. quadrifasciata*, originating from 16 different *Centaurea* species as well as from *Serratula tinctoria* and *Microlonchus salmanticus*. For *U. jaceana*, we have 51 records from three *Centaurea* species. From the two *Centaurea* species that are exploited by both *U. quadrifasciata* and *U. jaceana*, 88 samples of *C. jacea* and 34 samples of *C. nigra*, each containing between 50 and 200 flower heads respectively, were dissected during this survey and/or kept to assess the inhabitants that emerged from them.

RESULTS

Differences in life-history data of *U. jaceana* and *U. quadrifasciata*

Tables 1 and 2 summarize the data, which were obtained in our cage experiments with potted *C. jacea* and in the arena tests. These data show that *U. jaceana* females are larger, have about three times more eggs in their ovaries (Table 1) and deposit more eggs per visited bud than females of *U. quadrifasciata*. On average, *U. jaceana* females leave and revisit individual buds more often than *U. quadrifasciata* females. *Urophora jaceana* females are also quicker in their decision to probe a visited bud. The average time between first contact with a bud and the onset of probing is 2.05 min (median) (interquartile range = 0.82–6.50 min) in *U. jaceana* females and 4.07 min (2.68–11.69 min) in *U. quadrifasciata* females ($P = 0.033$, Mann-Whitney *U*-test). An important difference between the two *Urophora* species is that the average time on the bud required for laying an egg is about three times longer in *U. quadrifasciata* than in *U. jaceana* (Table 2). For *U. jaceana* females searching for oviposition sites, the temporal window during which a bud is acceptable is distinctly narrower than that for *U. quadrifasciata* (Table 1). *U. quadrifasciata* also profits from the

Table 2. *U. jaceana* and *U. quadrifasciata*: evaluation of video data

	U. jaceana	*U. quadrifasciata*	*P*
Time spent on a bud per deposited egg	1.91 (1.52–4.73) min (n = 18 females)	6.49 (5.23–7.91) min (n = 13 females)	0.0003
Number of visits per bud	4.0 (2.0–6.0) (n = 20 visited buds)	2.0 (1.0–2.0) (n = 23 visited buds)	0.0031
Duration of a visit to a bud	0.90 (0.23–3.54) min (n = 161 visits)	1.54 (0.55–1.98) min (n = 67 visits)	0.0001
Number of eggs deposited per visited bud	8.0 (2.5–15.25) (n = 20 visited buds)	2.0 (0.0–8.0) (n = 23 visited buds)	0.0094

Note: Summary of 14 (*U. jaceana*) and 15 (*U. quadrifasciata*) tests (duration of each test = 60 min) in cages in which *C. jaceana* buds (with stalk and 5–6 leaves) were offered. Results obtained with well-fertilized and unfertilized plants are pooled. The values give the median and the interquartile range. P = significance of Mann-Whitney U-test.

faster growth of its gall, a shorter larval period and the possibility of a second generation. Results obtained with hibernating galls, which were kept at 5°C before being transferred to room temperature in June/July, suggest that development after winter diapause (pupation period) is faster in *U. quadrifasciata* than in *U. jaceana* (Table 1). In both species, the adult males emerge on average 1–2 days before the females. When kept at 18–22°C, adults of the univoltine *U. jaceana* emerged from field-collected host material between April and July, with a distinct maximum in late May and early June (Fig. 1). The emergence pattern of the bivoltine *U. quadrifasciata*, whose host plants were kept under the same conditions, was irregularly spread from late August through the winter months until April (Fig. 1). These patterns suggest that the monophagous *U. jaceana* has a stringent synchronization with its hosts *Centaurea jacea* and *C. nigra*, whereas the oligophagous *U. quadrifasciata* is more flexible.

Effects of host plant quality

The effect of the nutrient regimes on the host plants

The two different nutrient regimes had no significant effect on the height of the plants (mean 41.9 *vs* 44.0 cm, n = 51 and 52 respectively) and the number of achenes per flower head, which varied considerably within and between plant individuals. In addition, the average dry weight of an achene (2.43 *vs* 2.44 mg) was almost identical and its nitrogen content (37.89 *vs* 38.67 mg $N \cdot g^{-1}$ dry weight) was only slightly changed in high or low nutrient regime plants. In comparison with the low nutrient group, the high nutrient plants developed significantly more side shoots, a more vigorous main stem (mean diameter 5.13 *vs* 4.31 mm, n = 35 and 34 respectively; $t = -4.33$, $P < 0.0001$), more flower heads (mean 20 *vs* 7, n = 50 and 53 respectively; $U = 246$, $P < 0.0001$) and more mature achenes per plant (mean 305 *vs* 124, n = 18 for both groups; $U = 93$, $P = 0.029$). In controls without oviposition by *Urophora*, a high nutrient supply also reduced the number of buds, which did not develop into flower heads. The proportion of aborted flower buds was 6.07% in the well-fertilized group and 17.42% in the unfertilized group (n = 36, $U = 94.5$, $P = 0.031$).

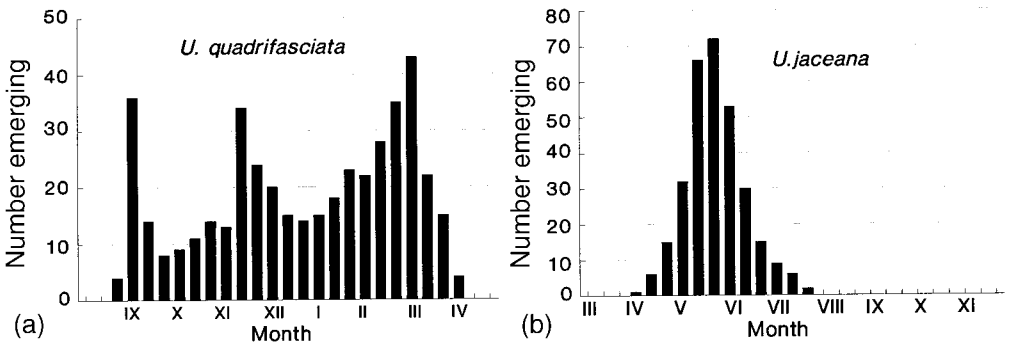

Fig. 1. Emergence patterns of *U. quadrifasciata* and *U. jaceana* from field-collected host material, which was stored at 18–22°C. Host plants of *U. quadrifasciata* = 11 samples from *Centaurea jacea*, *C. nigra*, *C. maculosa* and *C. scabiosa*. Host plants of *U. jaceana* = 9 samples from *C. jacea* and *C. nigra*.

Pre-alighting and post-alighting behaviour on C. jacea *plants*

Using walk-in cages, we assessed whether females of *U. jaceana* and *U. quadrifasciata* approaching the host plant in flight and landing on the test plants could discriminate between the two nutrient groups. In both species, there was no clear and consistent pre-alighting preference between well-fertilized and unfertilized *C. jaceana*. However, females of *U. jaceana* showed a weak preference for plants grown with the high nutrient supply (Table 3).

Once on the host plant (sequential choice tests), the females of both species spent on average 70% of their time examining the buds, probing and ovipositing. However, individual females were highly variable in their post-alighting searching and oviposition behaviour on the plants. Independent of the different fertilization treatments, *U. jaceana* females visited on average 35% and *U. quadrifasciata* females 20% of the available flower buds. The different treatments did not affect the time budget of the *Urophora* females – that is, the

Table 3. Visits to well-fertilized and unfertilized *C. jacea* by adult *Urophora*

Species tested	Sex	*n*	Unfertilized *vs* fertilized plants	$P(\chi^2$ test)
U. quadrifasciata	males	347	38 *vs* 54	0.095
U. quadrifasciata	females	354	49 *vs* 58	0.384
U. jaceana	males	250	32 *vs* 43	0.204
U. jaceana	females	250	35 *vs* 59	0.013

Note: Summary of results of 8 (*U. jaceana*) and 9 (*U. quadrifasciata*) tests in walk-in cages carried out between 20 July and 3 August 1994. Each individual fly that alighted on a test plant was treated as an independent data point and removed from the experiment. *n* = total number of individuals in the tests.

proportion of time devoted to different activities (resting, grooming, walking, probing, etc.) on the host plant.

Oviposition behaviour on flower buds of different quality

In the test series in which whole plants of both treatment groups were offered sequentially, females of *U. jaceana* chose a greater proportion of buds from the well-fertilized group for oviposition (Fig. 2a) (Wilcoxon-test, $Z = -2.3117$, $P = 0.0208$, $n = 12$), laid more eggs on well-fertilized plants (Fig. 2b) (Wilcoxon-test, $Z = -2.4318$, $P = 0.015$, $n = 12$) and laid significantly larger clutches into the buds of this group (Fig. 2c) (Wilcoxon-test, $Z = -2.7456$, $P = 0.0060$, $n = 12$). On well-fertilized plants, there was a highly significant positive correlation between clutch size and the time spent examining the bud, probing the bud and ovipositing (Spearman's rank correlation, $\rho = 0.890$, $P < 0.0001$, $n = 54$). In this group, the time costs per deposited egg were lower than on unfertilized plants [mean time required per deposited egg 0.98 min (range 0.83–2.20 min) *vs* 2.43 min (range 1.61–4.29 min); Wilcoxon-test, $Z = -2.0284$, $P = 0.0425$, $n = 7$]. Under the semi-natural conditions of this experiment,

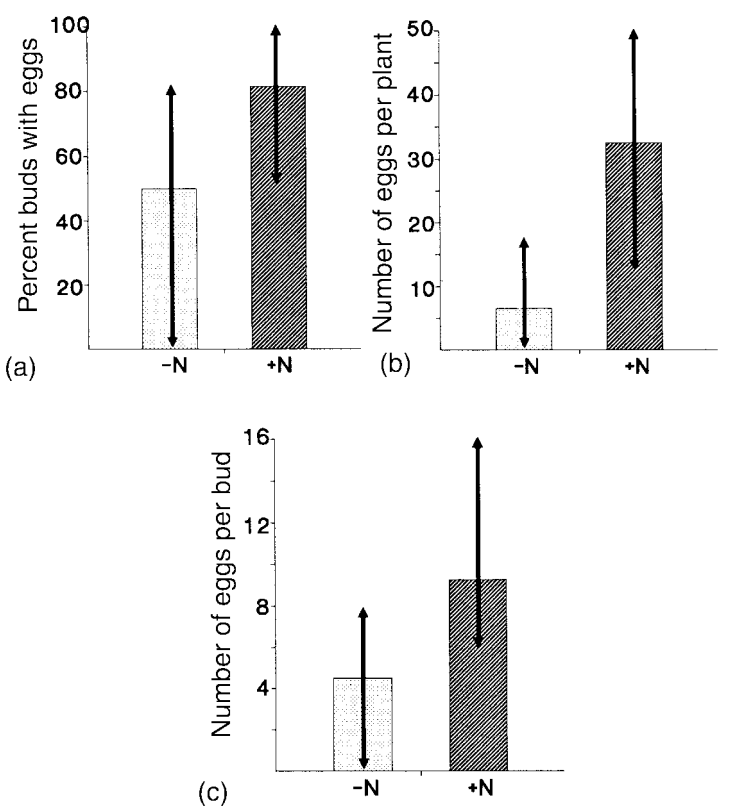

Fig. 2. Oviposition behaviour of *U. jaceana* females (median, interquartile range). (a) Percentage of the visited buds of acceptable size with eggs in unfertilized (–N) and well-fertilized (+N) *C. jacea* plants. (b) Number of eggs per plant for the unfertilized and well-fertilized groups. (c) Average numbers of eggs per bud on unfertilized and well-fertilized plants.

very few females of *U. quadrifasciata* visited buds, resulting in only five test series. Only three females showed probing and just a single female laid eggs (in both groups of test plants).

The choice tests with cut buds were analysed with the help of video recordings. Figure 3 allows a comparison of the two *Urophora* species for the relationship between the number of eggs laid per bud and the frequency of probing (as short probes usually did not result in oviposition, only probes which lasted more than 60 s were scored; the results obtained from unfertilized and well-fertilized plants were pooled). The regression with a slope of nearly 1 indicates that, in *U. quadrifasciata*, most cases of probing resulted in only one egg. *Urophora jaceana*, on the other hand, must have laid distinctly more eggs per probe. Figure 4 illustrates the influence of the treatment group of the host plant on the average time the females spent on the buds. Females of *U. jaceana* remained longer on the buds of well-fertilized plants (Wilcoxon test, $Z = -2.1658$, $P = 0.0303$, $n = 14$); those of *U. quadrifasciata* showed no preference ($Z = -0.2840$, $P = 0.7764$, $n = 15$). *Urophora jaceana* females revisited the buds of well-fertilized plants more often than those of unfertilized plants ($Z = -2.1181$, $P = 0.0342$, $n = 12$); no such preference was found in *U. quadrifasciata* ($Z = -0.0568$, $P = 0.9547$, $n = 15$).

In these choice tests, the proportion of 'motivated' females that investigated the buds was higher in *U. jaceana* (33.7%) than in *U. quadrifasciata* (18.6%) ($\chi^2 = 6.588$, $P = 0.0103$). An important difference between the two species was that only *U. jaceana* females preferred to oviposit into buds of the well-fertilized plants ($Z = -2.0004$, $P = 0.0455$, $n = 14$); the *U. quadrifasciata* females did not discriminate between the well-fertilized and unfertilized plants ($Z = -0.5923$, $P = 0.5536$, $n = 15$).

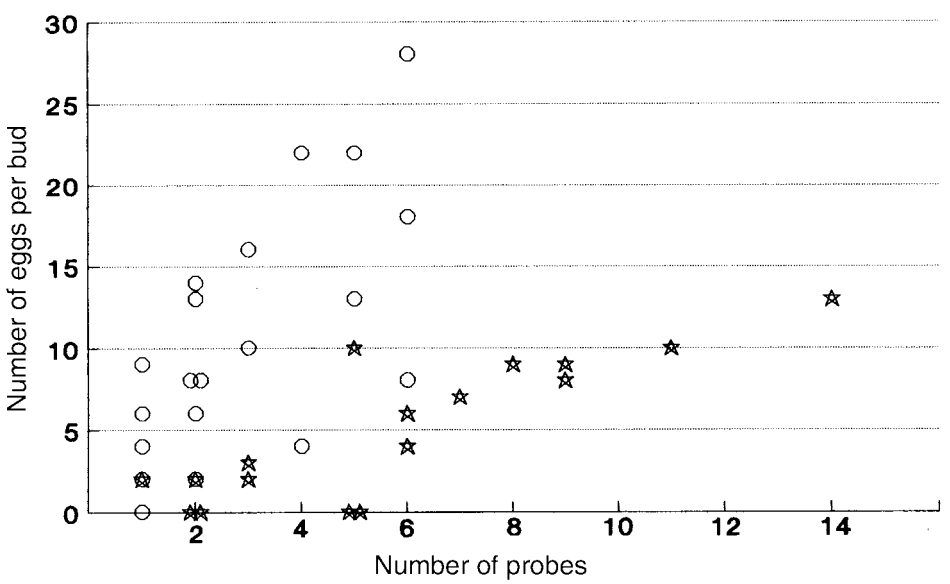

Fig. 3. Numbers of eggs per bud (pooled for unfertilized and well-fertilized plants) as a function of probing intensity (only probes lasting at least 60 s were included) for *U. quadrifasciata* (☆) and *U. jaceana* (○).

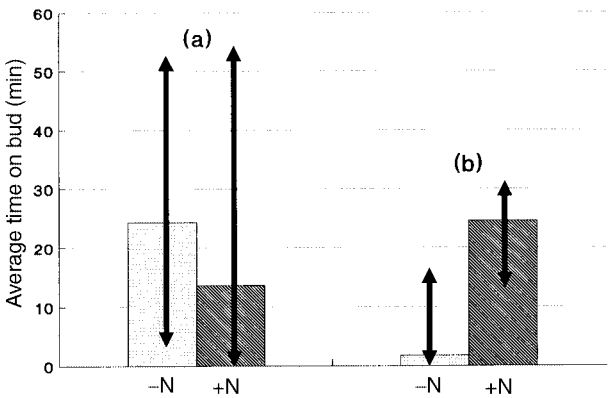

Fig. 4. Average time (median, interquartile range) spent on the buds of unfertilized (–N) and well-fertilized (+N) *C. jacea*: (a) *U. quadrifasciata*, (b) *U. jaceana*.

Larval development and reproductive fitness

The two nutrient treatments of the host plants had different consequences for the larval development of the two tephritid species. Male and female larvae of *U. jaceana*, the larger species, which induces a complex ovary-receptacle gall, had on average a higher body weight in well-fertilized than in unfertilized plants (males: $t = -2.50$, $P = 0.013$, $n = 191$ and 173; females: $t = -2.19$, $P = 0.029$, $n = 163$ and 130). In *U. quadrifasciata*, the species that forms a very simple achene gall, the larvae showed no increase in weight when developing in the buds of well-fertilized host plants.

Another difference between the two species was found in the impact of larval densities on larval weight. In female larvae of *U. jaceana*, even very high densities per occupied bud had no significant influence on the average weight in the flower heads of well-fertilized plants (one-factor ANOVA, $F = 1.340$, $P = 0.2700$). In the heads of unfertilized plants, however, densities of 15 and more larvae had a negative effect on larval weight (one-factor ANOVA, $F = 2.719$, $P = 0.048$) (Fig. 5). In males of *U. jaceana* and males and females of *U. quadrifasciata*, the two fertilization treatments had no effect on the relationship between larval densities and larval weight.

In both *Urophora* species, there was a strong positive correlation between larval fresh weight and dry weight of the adult stage in the plants of both treatment groups (unfertilized plants, *U. jaceana*: males $r = 0.969$, $P < 0.0001$, $n = 174$; females $r = 0.975$, $P < 0.0001$, $n = 96$; *U. quadrifasciata*: males $r = 0.805$, $P < 0.0001$, $n = 20$; females $r = 0.900$, $P < 0.0001$, $n = 31$). If the length of the hind tibia was taken as a measure of adult body size, there was a highly significant positive correlation between body size and the number of mature eggs in the ovaries in *U. jaceana* and a significant but less strong correlation in *U. quadrifasciata* (females at day 6: *U. jaceana*: $r = 0.757$, $P < 0.0001$, $n = 35$; *U. quadrifasciata*: $r = 0.507$, $P = 0.0043$, $n = 30$). Our results show that, for *U. jaceana*, the host plant quality is not only important in attracting ovipositing females and increasing larval weight, but can also have a positive influence on the reproductive fitness of the following generation.

Co-existence of *U. jaceana* and *U. quadrifasciata* in *Centaurea* populations

Although both *Urophora* spp. exploit a similar resource in the flower heads of *C. jacea* and *C. nigra*, a comparison of the observed and expected frequencies of simultaneous occupation of a flower head population by both species does not suggest any sign of interspecific competition. In our survey, 21 (23.9%) of the 88 *C. jacea* populations investigated were occupied by *U. jaceana* alone, 41 (46.6%) by *U. quadrifasciata* alone and 12 (13.6%) by both *Urophora* species. If the results obtained with 34 samples of *C. nigra* are included, the values become 39 (31.9%), 48 (39.3%) and 15 (12.3%) respectively. The percentages of observed simultaneous occurrences (13.6%, 12.3%) of both species correspond very well with the calculated values (11.2%, 12.6%) expected if the two species occur independently (13.6% *vs* 11.2%; Fisher's exact text, $P = 0.586$). Varley (1947) reported the simultaneous attack of the two *Urophora* species in his comprehensive census of a *C. nigra* stand near Cambridge. He found that *U. jaceana* was 'abundant' (more than 100 individuals per m^2) and *U. quadrifasciata* was 'common' (10–100 individuals per m^2) in this host plant population. The fact that Wadsworth (1914) and Dempster *et al.* (1995a), who studied the insect fauna of flower heads of *C. nigra* in North Wales and near Cambridge respectively, observed numerous *U. jaceana* but did not find *U. quadrifasciata*, might have been due to the temporal absence or a very low density of this species, as happened in our study area in 1985 and 1986.

In our samples, the presence or absence of one *Urophora* species in a flower head population did not influence significantly the intensity of attack by the other species. If samples with simultaneous attack are compared with samples attacked by a single *Urophora* species, neither the abundance of *U. quadrifasciata* was significantly reduced by *U. jaceana* ($F = 0.950$, d.f. = 1, $P = 0.336$) nor did *U. quadrifasciata* reduce significantly the abundance of *U. jaceana* ($F = 1.005$, d.f. = 1, $P = 0.327$). Myers and Harris (1980) found no negative correlation in numbers of *U. quadrifasciata* and *U. affinis* co-occurring in Canada, again suggesting that *U. quadrifasciata* can co-exist with a congener.

One marked difference between the two species in our study area near Bayreuth was that, over the last 20 years, *U. jaceana* was found continuously whereas *U. quadrifasciata* was not observed in either 1985 or 1986. It was, however, reared from *C. jacea* before these

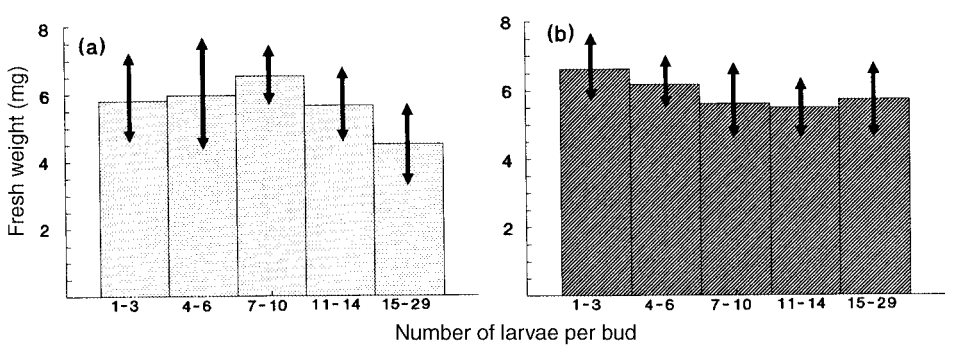

Fig. 5. Fresh weight (mg) (mean and 95% confidence interval) of mature female larvae of *U. jaceana* as a function of larval numbers per flower bud: (a) unfertilized plants (one-factor ANOVA, $F = 2.719$, $P = 0.048$), (b) well-fertilized plants (one-factor ANOVA, $F = 1.340$, $P = 0.270$).

years and reappeared later. From 1991 to 1995, it was a common species in *C. jacea* heads. Unstable dynamics of *U. quadrifasciata* are also reported from Canada, where the introduced populations occasionally declined sharply at some sites (Berube, 1980).

DISCUSSION

Apomorphic and plesiomorphic characters of *U. quadrifasciata* and *U. jaceana*

The tephritid genus *Urophora* is one of the few cecidogenous insect taxa, which allows a reconstruction to be made of the major steps in the evolution of its galls. The different *Urophora* species of the subgenus *Urophora* (*sensu* White and Korneev, 1989) present a sequence of stages of galls increasing in complexity (Zwölfer and Arnold-Rinehart, 1993). Of the *Urophora* species whose galls have been studied in some detail, *U. quadrifasciata* produces the most primitive gall (Arnold-Rinehart, 1989; Pönisch and Brandl, 1992; Baumann and Brandl, 1993). We assume that this species descended from an ancestor that was an internal seed feeder and that its type of gall can be taken as the starting point for the evolution of lignified single – and later multilocular – ovariole-receptacle galls (Zwölfer and Arnold-Rinehart, 1993). An interesting primitive character of *U. quadrifasciata* is that its larvae can develop only in pollinated *Centaurea* flower heads or flower heads also occupied by *U. affinis*, 'indicating a need for a pre-existing nutrient sink' (Harris and Shorthouse, 1996). In evolutionarily advanced *Urophora* species, such as *U. affinis* and *U. cardui*, which produce complex galls with lignified tissues, gall development is not dependent on pollination. As *U. jaceana* is both systematically and, with respect to its gall structure, very closely related to *U. affinis* (White and Korneev, 1989), we are sure that this species also can form galls in flower buds without pollination.

Compared with the plesiomorphic *U. quadrifasciata*, *U. jaceana* can be considered as a distinctly apomorphic form. This is not only shown by the morphogenesis and structure of its gall (Arnold-Rinehart, 1989) and a study of the salivary gland chromosomes (Pönisch and Brandl, 1992), but also by the fact that its first instar larva develops within the egg and hatches as a second instar (White and Clement, 1987). A similar behaviour is found in several other *Urophora* spp. that form multilocular block galls (*U. stylata*, *U. cardui*).

U. quadrifasciata: a primitive gall-maker but a successful colonizer

In spite of its modestly specialized status as a gall-maker, *U. quadrifasciata* is an ecologically and evolutionarily very successful species. It has a large original distribution area, it had a rapid rate of dispersal in regions where it has been introduced, it has a relatively broad host range and it is polytypic. According to White and Korneev (1989), *U. quadrifasciata* may even constitute a cluster of incipient species or siblings. In addition to its ability to switch to other host plant species and its remarkable potential for long-distance dispersal, *U. quadrifasciata* profits in comparison to other *Urophora* species from a faster rate of larval development and from the fact that its temporal window for oviposition is broader. One particular feature of *U. quadrifasciata* is its ability to produce a second generation. In spite of its much lower egg load (Table 1), this species has, under favourable conditions, a higher potential annual reproduction than the univoltine *U. jaceana* (Burkhardt, 1999).

Benefits and costs of a complex *Urophora* gall

To determine the selective advantages that have driven the evolution of complex galls and narrow ties with individual host plant species in the genus *Urophora*, we must look to the larval as well as the adult level.

If one compares *U. quadrifasciata* and its gall within a transformed achene with the *Urophora* species producing lignified, multilocular galls from ovaries and receptacle tissue of the flower head, it is obvious that the latter gall type supports the accumulation of more larval biomass. These *Urophora* species have a larger body size and, as far as we are aware, can produce more eggs per female than *U. quadrifasciata.* A prerequisite for the induction of the complex, lignified *Urophora* galls is the ability of the young larvae to generate a powerful metabolic sink. The advantage of this evolutionary innovation is the profit from additional nutrients supplied via a newly formed vascular system (Harris and Shorthouse, 1996). In the buds of well-fertilized plants, larvae and adults of *U. jaceana* had, on average, a significantly higher body weight and females produced more eggs than those which developed in unfertilized hosts. That the growth of larvae was influenced by the nutrient regime of the host plant in *U. jaceana* but not *U. quadrifasciata* can presumably be explained by the different ways they secure resources.

The lignification of the gall of *U. jaceana* offers an additional advantage, as it provides its inhabitants with a protective cover. Schlumprecht (1990) showed that large lignified *Urophora* galls offer a partial refuge for the larvae, as the females of parasitoids, such as *Eurytoma* species, are constrained by the length of their ovipositor. He calculated that, in *U. jaceana*, this refuge effect against parasitism begins with galls of more than six chambers (Zwölfer and Arnold-Rinehart, 1994).

These benefits are connected with time costs: the development of the gall of *U. quadrifasciata* is complete after only 21 days, whereas *U. jaceana* galls require about 28 days and other *Urophora* species with multilocular galls need 30 or more days (Arnold-Rinehart, 1989).

In choice tests, *U. jaceana* females showed a slight pre-alighting preference for the vigorous plants grown with a high nutrient supply. Therefore, it is possible that they already recognize the quality of their host plants at a distance. When in close contact with the host – that is, when confronted with *C. jacea* buds – *U. jaceana* females distinctly preferred to oviposit into the flower heads of well-fertilized plants. On average, they laid 6.5 eggs (= 3.3% of their egg load) on unfertilized plants and 32.5 eggs (= 15.4% of their egg load) on well-fertilized plants. In *U. jaceana*, the ovipositional preference for plant quality corresponds with a tolerance of high larval densities. In heads of well-fertilized plants, the average larval fresh weight was independent of the number of larvae per bud (Fig. 5). In heads of unfertilized plants, a density of 15 or more larvae per bud led to a significant decline in larval weight.

The behaviour of *U. jaceana* resembles that of *U. cardui*, another *Urophora* species with a complex lignified gall, whose females can adjust their clutch size in the same way to the quality of the substrate (Freese and Zwölfer, 1996). Others have observed similar behaviours. Janz and Nylin (1997) showed that, among species of nymphalid butterflies with different host ranges but with one host in common, the specialists made fewer errors in relation to host plant quality than did the relative generalists (Bernays, 1998). Similarly, De Bruyn (1994), in his comparison of the life-cycle strategies of *Lipara* spp. on *Phragmites australis*, found that the females of specialized gall makers (*L. lucens* group), but not those

of the less-specialized *L. pullitarsis*, were able to discriminate between host shoots of different quality. That the ovipositional decisions made by the females have consequences for the fitness of the offspring of gall formers has been shown for the tephritid *U. cardui* (Freese and Zwölfer, 1996) and the cynipid gall wasp *Cynips divisa* (Gilbert *et al.*, 1994).

An interesting result of our oviposition tests was the difference in time costs per deposited egg for *U. jaceana* and *U. quadrifasciata*. *Urophora quadrifasciata* females laid their eggs usually singly and needed on average three times longer to deposit an egg than *U. jaceana* females, which lay clutches. Moreover, in *U. jaceana*, the decision to lay eggs was distinctly quicker on well-fertilized plants, as the average time required for a female to deposit an egg on such buds was halved. The deposition of clutches and the greater decisiveness may give the specialist *U. jaceana* an advantage in reducing predation risk when they are handling flower buds.

We conclude that, in *U. quadrifasciata* and *U. jaceana*, the different ovipositional decisions of the females (i.e. the selection of the host species, the oviposition site and the clutch size) correspond with the different abilities of their larvae to extract food from their host plant. The specialist *U. jaceana* compensates for its dependence on a much reduced host plant spectrum and higher developmental time costs by its efficiency of exploitation of its larval resource. It makes it a successful exploiter of local populations of *C. jacea* and *C. nigra*, as long as the circumstances of the host plant remain more or less stable. In unpredictably variable environments, the generalist and pioneer species *U. quadrifasciata*, with its faster larval development, a second generation per annum and a much higher dispersal capacity, is the more successful species.

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