Comparative plant-herbivore interactions involving willows and three gall-inducing sawfly species in the genus *Pontania* (Hymenoptera: Tenthredinidae)[1]

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Abstract: The ovipositional preferences of female *Pontania* species, which induce leaf galls on willows, were examined in relation to the performance of progeny in terms of larval establishment and survival in galls. Three *Pontania* species separated widely in geographic location were studied: *Pontania sp.* 1 on *Salix scouleriana* near Flagstaff, Arizona, U.S.A., *Pontania amurensis* on *S. miyabeana* near Sapporo, Hokkaido, Japan, and *Pontania pustulator* on *S. phylicifolia* in eastern Finland, where two populations were examined. The species are uncommon to rare in the locations studied and depend on disturbance resulting in populations of young willows or young ramets. All species showed a strong and significant ovipositional preference for longer shoots and younger trees or ramets; older plants were not attacked. Survivorship differed considerably between species: *Pontania sp.* 1 had 80% of galls aborted, low parasitism and 16% survival, *Pontania amurensis* had few galls aborted, moderate parasitism and 70% survival, and *Pontania pustulator* was exposed to heavy parasitism and had a mean of 27% survival for the two populations. The pattern of ovipositional preference was not strongly related to probability of larval establishment in a gall for any of the populations studied. Only *Pontania sp.* 1 showed a weak but significant relationship between the pattern of preference and larval survival. The apparent weak linkage between preference and performance may be accounted for by four alternative hypotheses. *i)* Females are so selective that all galls initiated are situated on high-quality resources: females are specific to one species of willow, they are highly selective for very young plants, and on these plants they prefer the longer shoot-length classes. *ii)* Low predictability of larval survival negates any benefits of a preference by females. *iii)* Preference evolves independently of performance because young plants and long shoots provide better resources in which females oviposit. *iv)* Young leaves, on which galls are initiated and larvae develop, are a relatively uniform, nutritious and predictable resource, such that there is a broad range of shoot lengths similarly suitable for larval survival. The results provide insights into the factors contributing to the rarity of these sawflies, their highly patchy distribution over the landscape, and their population dynamics.

Keywords: gall-inducing sawflies, plant-herbivore interactions, *Pontania*, preference-performance linkage, *Salix*.

Résumé: Les auteurs ont examiné la relation entre les préférences affichées par les femelles prêtes à pondre de diverses espèces de *Pontania* provoquant la formation de galles foliaires chez le saule et deux variables liées au rendement de la progéniture, soit le taux d'établissement et le taux de survie des larves dans les galles. Trois espèces vivant dans trois régions très éloignées les unes des autres ont été étudiées : *Pontania sp.* 1 sur *Salix scouleriana*, près de Flagstaff, Arizona, États-Unis; *P. amurensis* sur *S. miyabeana*, près de Sapporo, Hokkaido, Japon; *P. pustulator* (deux populations) sur *S. phylicifolia*, dans l'est de la Finlande. Ces espèces sont peu communes à rares dans les zones d'étude, et leur abondance fluctue en fonction des perturbations environnementales induisant la formation de peuplements de jeunes saules ou de jeunes ramets. Chez les trois espèces, les femelles prêtes à pondre ont affiché une préférence marquée et significative pour les pousses plus longues et les arbres ou les ramets plus jeunes. Les saules plus âgés n'ont pas été attaqués. Les taux de survie ont varié considérablement d'une espèce à l'autre : *Pontania sp.* 1, taux d'avortement de la galle de 80 %, taux de parasitisme faible et taux de survie de 16 %; *P. amurensis*, avortement limité à quelques galles seulement, taux de parasitisme modéré et taux de survie de 70 %; *P. pustulator*, taux de parasitisme élevé et taux de survie moyen de 27 % parmi les deux populations étudiées. Aucune relation étroite entre les préférences liées à la ponte et la probabilité d'installation de la larve dans une galle n'a été observée chez aucune des populations étudiées. Seul *Pontania sp.* a montré une relation faible mais significative entre les préférences liées à la ponte et la survie de la larve. La faiblesse de la relation entre les préférences manifestées par les femelles à l'égard de l'hôte et le rendement de la progéniture pourrait s'expliquer par une ou l'autre des quatre hypothèses suivantes : *i)* les femelles font preuve d'une sélectivité telle que toutes les galles se trouvent sur des ressources de qualité supérieure : elles affichent une spécificité à l'égard d'une espèce de saule et une grande sélectivité à l'égard des très jeunes plants, chez qui elles recherchent de préférence les pousses les plus longues; *ii)* la faible prévisibilité du taux de survie de la larve annihile tous les avantages découlant des préférences affichées par les femelles; *iii)* les préférences des femelles évoluent sans égard au rendement de la progéniture, car les jeunes plants et les pousses plus longues leur procurent de meilleures ressources; *iv)* les jeunes feuilles, sur lesquelles se forment les galles qui abriteront les larves durant leur développement, constituent une ressource de qualité relativement uniforme, nutritive et prévisible, si bien qu'il existe un large éventail de longueurs de pousses favorisant la survie larvaire. Les résultats obtenus dans le cadre de cette étude laissent entrevoir les facteurs expliquant la rareté de ces tenthrèdes, ainsi que leur répartition hautement agrégée dans le paysage et la dynamique de leurs populations.

Mots-clés: tenthrèdes galligènes, interactions plantes-herbivores, *Pontania*, relation entre préférence et rendement, *Salix*.

Introduction

Linkage between ovipositional preference for particular plant species or plant parts in relation to larval performance on the plant or module where the egg was laid has received much attention in recent years. The hypothesis is that females of a species should evolve with the capacity to make effective choices among plants or modules of plants such that the collective performance of her larvae is maximized and the female's fitness is maximized. Larval performance may be measured as survival, growth rate, feeding efficiency, or fecundity of the resulting adult female. The concept can be traced back to Darwin (Thompson & Pellmyr, 1991). Since Singer (1986) emphasized the importance of this relationship and outlined the methodologies that might be employed to study it, three influential reviews (Thompson, 1988; Courtney & Kibota, 1990; Thompson & Pellmyr, 1991) have suggested that the hypothesis on preference-performance linkage is not supported in all studies. Empirical studies have generated a debate about circumstances under which natural selection will work to maximize or minimize the role of a preference-performance linkage in herbivorous insects (Price, 1997).

One approach to understanding the range of preference-performance linkage from high to absent involves the Phylogenetic Constraints Hypothesis (Price, 1994). The prediction is that ancient evolved morphological and life history constraints define the selectivity of a female in her ovipositional choices. For example, the saw-like ovipositor of a sawfly is adaptive for piercing plant tissue and ovipositing within the plant. Such an intimate relationship between plant and sawfly is likely to result in highly selective and adaptive behavior favoring optimal plant modules which minimize wear on the ovipositor and maximize both ease of oviposition and larval performance. Hence, under the long-term influence of such phylogenetic constraints, we should anticipate a high preference-performance linkage. Note that the prediction concerns the very precise utilization of particular plant species and particular module quality within that species. These highly refined preferences contrast with the kinds of ovipositional choices seen in most insects for general habitat types or ovipositional niches, more like the species to be considered next. In contrast to those insect herbivores with high preference-performance linkage, a lineage may evolve with a constraint in which eggs are laid late in one season, overwintering occurs as an egg or larva, but larval feeding is initiated on early foliage many months after oviposition. The selective feedback from larval performance to female preference will be weak or absent. Then we should not expect much or any female ovipositional preferences in relation to larval performance. Indeed many females of such species, as in the Lepidoptera, oviposit on trunks of trees, stones, or on late season foliage irrelevant to larval nutrition (cf. Price, 1997 and references therein). There may well exist a gradient of preference-performance linkage in nature, from very strong linkage to no linkage, and the discovery of such a pattern and the understanding of mechanisms determining the pattern are of central concern for the development of broadly based mechanistic and factual theory on plant and herbivore interactions (Price, 1996; as opposed to hypothetical theory).

In relation to these hypotheses on preference-performance linkage and phylogenetic constraints, our research group has focused on sawflies in the family Tenthredinidae which illustrate an apparently wide range in preference-performance linkage within a single phylogenetic lineage, containing species with the same phylogenetic constraints. Some sawflies, that form galls on willows illustrate the strongest preference-performance linkage described to date: in Arizona, U.S.A. (Craig, Itami & Price,1989), on Hokkaido, Japan (Price & Ohgushi, 1995), and in North Karelia, Finland (Price, Roininen & Tahvanainen, 1987a, b; 1997; Roininen, Price & Tahvanainen, 1993). These examples involve stem- and bud-galling species in the genus *Euura* and a leaf-edge gall inducer in the genus *Phyllocolpa*. However, in a species involving the leaf-galling genus *Pontania*, a preference-performance linkage was not found in one study (Clancy, Price & Sacchi, 1993), but was discovered in another study on the same species (Stein & Price, 1995). Hence, to further understanding of preference-performance patterns, we undertook the comparative study described in this paper, concentrating on three *Pontania* species not studied before.

We asked the following questions: (*i*) In the genus *Pontania*, which forms galls on leaves of willows, are there similarities in the plant-herbivore relationship in widely separated species in Arizona, U.S.A., Hokkaido, Japan, and North and South Karelia, Finland? (*ii*) Is there evidence of a preference-performance linkage in each of the species studied? (*iii*) What constraints act on the development of a preference-performance linkage? We submit that this strongly comparative approach on species within a single genus, widely separated in geographic location, contributes significantly to the discovery of pattern in nature and the mechanisms driving the pattern. The study also advances comparative studies on the related genera, *Pontania*, *Euura*, and *Phyllocolpa* (Price, Clancy & Roininen, 1994; Price, Craig & Hunter, 1998).

Our general approach is to use the modular structure of the host plants as the independent variable for examining preferences of females and performances of larvae, especially using pattern in relation to shoot length variation. Thus, we continue with a long-standing tradition in plant-herbivore studies employing plant modules as important host characters to which herbivores respond. For example, the modular structure of woody plants has been emphasized in studies of birch species in relation to herbivores (Niemelä & Haukioja, 1982; Haukioja *et al.*, 1990; Senn, Hanhimäki & Haukioja, 1992) and moose browsing on birches and pines (Danell & Huss-Danell, 1985; Bergström & Danell, 1987; Danell & Bergström, 1989), while leaf size is a commonly studied variable in relation to leaf-miner patterns of attack and survival (Simberloff & Stiling, 1987; Faeth, 1991; Auerbach, Connor & Mopper, 1995). Since Harper (1977; 1981) emphasized the importance of modular structure and "the plant as a population of parts" (Harper, 1977, p. 20), increasing awareness has developed in relation to herbivores utilizing plants and impacting plants, but much more emphasis is needed on the involvement of structural and functional mechanisms of plants in the development of hypotheses and theories on plant-herbivore interactions (Haukioja *et al.*, 1990; Haukioja, 1991). This study contributes to the development of such hypotheses.

Materials and methods

STUDY ORGANISMS

The three genera of gall-inducing sawflies are *Pontania*, the leaf-lamina gallers, *Phyllocolpa*, the leaf-edge gallers, and *Euura*, the petiole, midrib, bud, and stem gallers. One species in both *Pontania* and *Phyllocolpa* and seven species of *Euura* have shown strong preference-performance linkage, with each species' relationship described in published papers (see Price, Craig & Hunter, 1998, for references). Therefore, the addition of three species of *Pontania* in this paper considerably broadens the scope of our comparisons.

Pontania species are generally specific to a single host plant species and each interaction develops a gall with distinctive morphology. Thus, even though a specific name is not available for one of the species we studied, it can be distinguished in the future by host and gall morphology. In Arizona we studied *Pontania* sp. 1 on *Salix scouleriana* Barratt; on Hokkaido, *Salix miyabeana* Seeman was attacked by *Pontania amurensis* Zinovjev; in Finland *Pontania pustulator* Forsius attacked *Salix phylicifolia* L. The three species initiate relatively large galls compared to most species in the genus and are illustrated in Figure 1. *Pontania amurensis* is not a well-known species, but was described by Zinovjev in 1985.

Pontania sawflies emerge in the spring and oviposit on very young leaves of willows at the tips of rapidly developing shoots. The female induces gall formation and larvae feed within the gall until fully grown, emerge from the gall, and spin a cocoon in the soil in which they overwinter. Pupation occurs in the following spring and adults emerge. There is one generation per year and galls are present on plants for most of the period in which leaves are present, enabling easy detection and sampling. Females oviposit into the very youngest and smallest leaves on a shoot and leaves subsequently expand to full size as the galls develop. Therefore, leaf size cannot be used by females in decisions on where to oviposit; they probably respond to phenolic

Pontania sp. 1   *Pontania amurensis*   *Pontania pustulator*

FIGURE 1. Illustrations of the three *Pontania* gall-forming species studied, at the same scale.

glucoside content of young leaves, as in the only two gall-forming sawflies studied in detail (Kolehmainen *et al.*, 1994; Roininen *et al.*,1999).

The architectural development of willow plants is fundamental to an understanding of how we have approached studies on gall-inducing sawflies. Many of our investigations have focused on how willow plants grow, the responses of sawflies to patterns of growth and their consequences related to distribution and abundance of sawflies (Craig, Price & Itami, 1986; 1989; Fritz *et al.*,1987; Price, Roininen & Tahvanainen, 1987a,b; Price, Craig & Roininen, 1995; Price *et al.*, 1995; Price, Roininen & Tahvanainen, 1997; Price, Roininen & Carr, 1997; Price, Craig & Hunter, 1998; Price, Roininen & Zinovjev, 1998; Preszler & Price, 1988; Price, 1989; 1991). Underappreciated generally in the literature of plant-herbivore interactions is the extraordinary predictability of modular development in woody plants to which herbivores respond (Price, 1991; Price *et al.*, 1995). Young plants or ramets produce long shoots and shoot length declines with age. Long shoots have a greater diameter, larger leaves, longer internodes, and grow more rapidly and for longer in the season than shorter shoots and have a lower probability of abscission (Price, Roininen & Tahvanainen, 1987a; Craig, Itami & Price, 1989; Price, Roininen & Carr, 1997). In addition, longer shoots contain higher concentrations of phenolic glucosides than shorter shoots (Price *et al.*, 1989). All these traits correlated with shoot length make this variable a very useful synoptic index that can be applied broadly (Price *et al.*, 1995). For the species studied, however it is impossible to tell without detailed studies which trait is the key for understanding ovipositional choices and larval survival.

STUDY SITES

i) The Arizona site was situated on the San Francisco Peaks about 12 km north of Flagstaff on Snowbowl Road at approximately 2790 m above sea level (35° 20' N, 111° 43' W). The host plant, *S. scouleriana*, is distributed widely on rocky slopes in the area, but this was the only site at which this *Pontania* species was found. The site had been highly disturbed by bulldozing and the shrubby damaged willows responded by developing rapidly growing new shoots from their bases. The population was studied in 1996. *ii*) On the northern island of Hokkaido, Japan, *S. miyabeana* was growing in a highly disturbed landfill site just west of Sapporo, near the Ishikari River at about 10 m above sea level (43° 10' N, 141° 23' E). This population was studied in 1993. *iii*) In eastern Finland, two populations of *S. phylicifolia* were found at sites disturbed from agriculture, where young shrubs had colonized. The Rantakylä site was located near Joensuu, North Karelia (62° 37' N, 29° 50' E) at an elevation of approximately 85 m above sea level. The other site was near Simpele, South Karelia (61° 28' N, 29° 27'E) and 75 m above sea level. Sampling occurred in 1995.

In all cases, the populations of *Pontania* were small and isolated, occurring on very young shrubs or highly disturbed shrubs, or on young trees in the case of *S. miyabeana*. These species rarely occur at population densities high enough to provide large sample sizes.
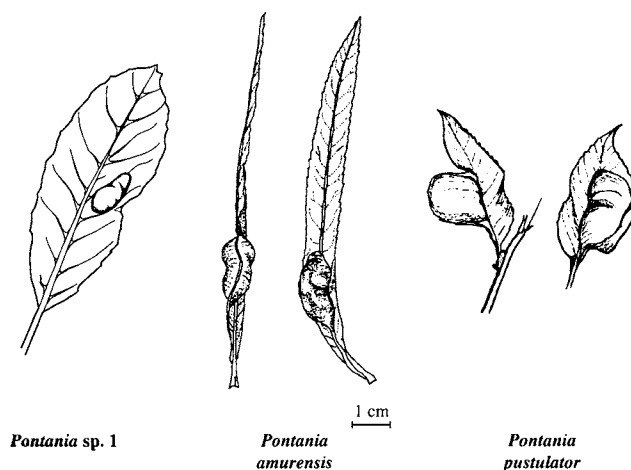
Methods

At all sites we used identical methods to sample most of the plants that were attacked by sawflies. All plants at a site were within cruising range of a single female sawfly, making it possible to evaluate the selectivity of females. For the tree species, *S. miyabeana*, we measured shoot length of all shoots per sample tree. The shrubby willows, *S. phylicifolia* and *S. scouleriana*, are clonal, sending up stems or ramets from a basal root stock, and on any ramet sampled, all shoots were measured. In every case the number of years a tree or ramet had grown was recorded, based on the nondestructive method of counting the number of winter bud scars up each stem (Craig, Price & Itami, 1986, for details). For each shoot the number of galls was recorded, and for a subsample the number of leaves per shoot was counted. All galls were opened and classified as follows: (*i*) Larva survived, meaning that it had emerged from the gall or was about to emerge; (*ii*) Larva dead, either from parasitic wasp or other insect attack or from unknown causes (the approximate time of death was divided into early, mid and late larval stages depending on the relative size of the larval remains), (*iii*) Larva established, signified by evidence of larval feeding with fecal pellets evident in the gall, and (*iv*) Aborted, meaning no evidence that an egg had hatched in the gall, implicating a plant resistance factor which caused either death of the egg or the female initiating the gall to withhold an egg. These two possible factors resulting in an aborted attempt to establish a larva in a gall could not be distinguished at the time of sampling.

At the Flagstaff site, 22 genets (*i.e.*, distinct genotypes) were sampled and 73 ramets, for a total of 696 shoots and 464 galls. At Sapporo, the main study site provided 10 genets, 484 shoots and 154 galls. At an adjacent site with larger trees, most had no galls ($n = 10$) and on one tree we subsampled a branch with 254 shoots and 20 galls. The Rantakylä site yielded 12 genets, 16 ramets, 647 shoots, and 57 galls. The Simpele site provided a sample of 10 genets, 16 ramets, 674 shoots, and 35 galls.

These data enabled us to address questions (*i*) to (*iii*) in the introduction. We compared patterns of attack and survival in relation to shoot length of the plants utilized and estimated the strength of any preference-performance linkage of the species in question. Then we developed hypotheses on why the preference-performance linkage may be strong or weak, depending on the survival and mortality data collected.

Because time of death and cause of death could be estimated, comparable survivorship curves for each population were developed. Starting with a standardized cohort of 100 galls we estimated the percent surviving from galls to early, mid and late larvae to survivors either emerged from the gall or about to emerge. Thus, the two main causes of loss from the cohort occurred between galls and early larva as an 'aborted' category and between mid and late larva as a 'parasitized' category. In fact, the parasitized category included mortality caused by true parasitic wasps, or parasitoids, and also species that killed the *Pontania* larva and fed on gall tissue. The latter are often referred to as 'inquilines', which in the cases studied included chalcid wasp larvae and moth larvae.

For ease of comparison among the four populations studied, we portray patterns of probability of attack per shoot, probability of establishment of a larva in a gall per gall formed, and probability of survival per gall formed. All are presented as simple linear regressions, with shoot length as the independent variable. We also plot the relationship between tree or ramet age in years and the number of galls per tree or ramet. We designate the populations by their locality in each figure as Flagstaff for *Pontania* sp. 1 on *S. scouleriana*, Sapporo for *Pontania amurensis* on *S. miyabeana*, and Rantakylä and Simpele for the two populations of *Pontania pustulator* on *S. phylicifolia* in Finland.

Because sawfly populations and the populations of vigorously growing plants are so small, we measured all shoots on each of the plants sampled. This was the only way in which we could obtain adequate replication per shoot-length class to enable discovery of any patterns in preference and performance. This raises the question of pseudoreplication (Hurlbert, 1984), because shoots on one plant are not independent of each other. Sampling in this manner has been of long-term concern in our studies and is addressed most explicitly in Price *et al.* (1995), with the following mitigating considerations: (*i*) Obtaining one shoot sample per plant genotype would require several hundred plants for an adequate sample, which were not available in the study of these *Pontania* species. (*ii*) A female sawfly cruising among shoots and making ovipositional decisions makes choices based on individual shoot tips, and each decision may be considered as an independent act. Therefore, shoots on the same plant may provide independent samples. This is not quite true because females grade responses to shoot length, evaluating shoots in a relative manner (Craig, Itami & Price, 1989), but the female's decisions render a view that shoots on the same plant are treated more or less independently by a cruising population of sawflies. (*iii*) We have not designed experiments, the butt of Hurlbert's (1984) chief concern and, if we are to study these rare kinds of insects, we must accept the limitations that nature imposes. In general, we are confident that the sampling methods employed are the best available and the results and biological relevance are undistorted.

Results

PLANT AGE AND SHOOT LENGTH

In each of the host plant species studied over 50% of shoots were in the shortest two shoot-length classes (0-9.9 cm), while long shoots were relatively rare in the population of shoots (Table I). For example, in *S. miyabeana*, 64% of shoots were less than 10 cm long, but only 4.4% of shoots were 25 cm or more. Number of leaves in each shoot-length category was also strongly skewed toward the shortest classes, with more than 50% of leaves available in the shortest three classes (0 -14.9 cm). Leaves are the resource utilized by *Pontania* species, so it is important to note the high percentage of leaves in the short shoot-length classes and the very low percentage of leaves in the long shoot-length classes.

All *Pontania* species showed a strong preference for the longest shoots available (Table II), and consequently for the younger trees or ramets (Figure 2). For each species there was a general trend for the mean number of galls per ramet or tree to decrease as the age and/or size of host plants increased. In all three species and four populations a strong and significant preference to attack longer shoot-length classes was observed (Table II, Figure 3).

TABLE I. Distribution of shoots and leaves in each shoot-length class for the three species of willow studied. R and S indicate the Rantakylä and Simpele populations, respectively

Shoot-length class (cm)	Salix miyabeana Shoots (%)	Salix miyabeana Leaves (%)	Salix phylicifolia Shoots (%) R	Salix phylicifolia Shoots (%) S	Salix phylicifolia Leaves (%) R	Salix phylicifolia Leaves (%) S	Salix scouleriana Shoots (%)	Salix scouleriana Leaves (%)
0–4.9	44	31	42	31	36	24	50	27
5–9.9	20	18	33	24	32	21	18	17
10–14.9	15	17	12	13	13	12	6	7
15–19.9	9	12	5	7	6	7	4	6
20–24.9	7	13	3	6	4	7	3	5
25–29.9	3	6	2	4	3	5	5	9
30–34.9	0.8	1	1	3	2	4	3	6
35–39.9	0.4	0.8	0.6	3	1	4	2	4
40–44.9	0	0	0.3	3	0.5	5	2	4
45–49.9	0.2	0.5	0.3	1	0.6	2	0.9	2
50–54.9			0.3	0.4	0.6	0.7	1	3
55–59.9			0.2	1	0.4	2	0.4	1
60–64.9			0.3	0.4	0.7	0.8	0.4	1
65–69.9			0	0.3	0	0.6	0.3	1
70–74.9			0.3	0.9	0.7	2	0.4	2
75–79.9			0	0.3	0	0.7	0	0
80–84.9			0	0	0	0	0.1	0.5
85–89.9			0.2	0.6	0.6	1	0.4	2
90–94.9				0.1		0.2	0.3	1
95–99.9				0.1		0.3	0.1	0.5
100–104.9				0.3		0.8	0	0
105–109.9				0		0	0	0
110–114.9				0.3		0.8	0.1	0.6
115–119.9				0		0	0.3	1
120–124.9				0		0		
125–129.9				0.1		0.3		

However, the fate of progeny once galls were initiated differed considerably among species as indicated in their survivorship curves (Table II, Figure 4). Establishment of feeding, early larvae in galls was very high, over 90%, in the Sapporo and the two *P. pustulator* cohorts (Rantakylä and Simpele), but aborted galls were common in the Flagstaff cohort. Conversely, the Flagstaff cohort was hardly parasitized, while the Sapporo population showed moderate levels of attack and the *P. pustulator* cohorts were heavily attacked. Thus, in terms of a preference-performance linkage, each sawfly species was exposed to different kinds and levels of mortality factors and overall survivorship.

With respect to performance of progeny, a female should have the strongest potential influence on the minimization of abortion and the maximization of larval establishment. This is because larval establishment depends on the intrinsic, direct interaction between plant and herbivore to which the herbivore population can adapt without the
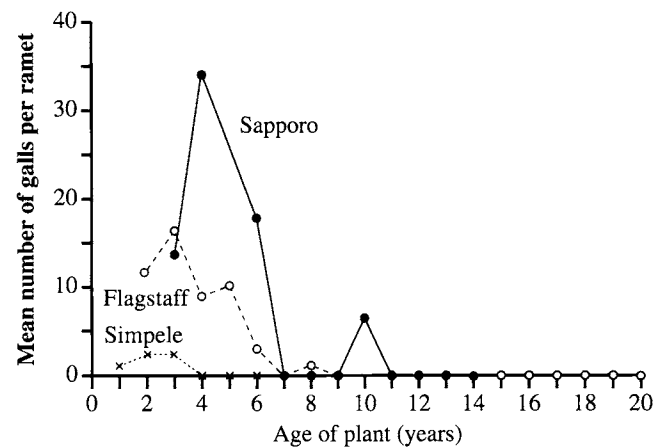

FIGURE 2. Distribution of galls in relation to tree or ramet age showing a strong preference for young plants. *Pontania pustulator* at the two sites studied showed similar distributions, so only the Simpele data are shown.

TABLE II. Summary of statistical tests on the preference-performance relationship in the three species of *Pontania* and four populations studied

Population	*Pontania* species	Number of shoots/ galls sampled	Regression equation[a]	Number of shoot length classes	r^{2}[b]	Significance
PROBABILITY OF ATTACK PER SHOOT LENGTH CLASS:						
Flagstaff	*Pontania sp.* 1	696/464	$Y = 0.26 + 0.01X$	14	0.58	$p < 0.05$
Sapporo	*Pontania amurensis*	484/154	$Y = 0.21 + 0.02X$	6	0.67	$p < 0.05$
Rantakylä	*Pontania pustulator*	647/57	$Y = 0.02 + 0.01X$	10	0.80	$p < 0.01$
Simpele	*Pontania pustulator*	674/35	$Y = 0.01 + 3.33E - 3X$	13	0.79	$p < 0.01$
PROBABILITY OF ESTABLISHMENT OF FEEDING LARVAE IN GALLS:						
Flagstaff			$Y = 0.10 + 1.68E - 3X$	13	0.29	N.S.[c]
Sapporo			$Y = 0.88 + 2.72E - 3X$	6	0.09	N.S.
Rantakylä			$Y = 1.04 - 3.18E - 3X$	9	0.51	$p < 0.05$
Simpele			$Y = 0.97 - 2.50E - 4X$	11	0.00	N.S.
PROBABILITY OF SURVIVAL OF PROGENY TO EMERGENCE FROM THE GALL:						
Flagstaff			$Y = 0.04 + 1.81E - 3X$	13	0.43	$p < 0.05$
Sapporo			$Y = 0.86 - 0.01X$	6	0.34	N.S.
Rantakylä			$Y = 0.21 + 5.87E - 4X$	9	0.00	N.S.
Simpele			$Y = 0.22 + 8.72E - 4X$	11	0.01	N.S.

X is the independent variable, shoot length class, and Y is the dependent variable, probability of attack, probability of establishment, or probability of survival.

[a]Slopes of regression equations are very low in many cases and are expressed in these cases as E – 3X or E – 4X meaning an exponent of –3 or –4.

[b]r^{2} is the approximate value of the amount of variance accounted for by the regression equation.
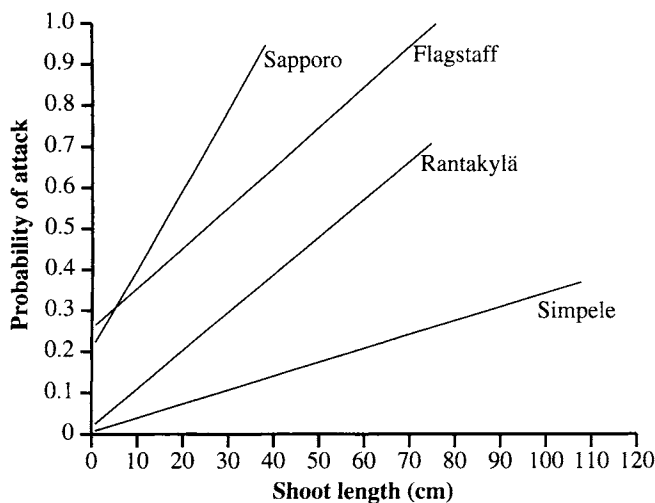
[b]N.S. indicates not significant.

FIGURE 3. Probability of a shoot being attacked by *Pontania* species in relation to shoot length. All regressions are significant and equations are provided in Table II.

FIGURE 4. Survivorship curves for cohorts from the four populations of Pontania studied.

influence of additional extrinsic selective pressures, especially involving mortality caused by parasitic wasps and inquilines. However, in the three *Pontania* species and the four populations, we found no positive significant correlation between probability of larval establishment and shoot length, and therefore no positive linkage between this aspect of female preference and larval performance to the early larval stage (Figure 5). The Flagstaff population showed a response very close to significance ($r = 0.54$, critical value for $p < 0.05$ is 0.55). The only significant relationship was negative, involving the Rantakylä population. In general, no matter which shoot-length class is selected by ovipositing females, larval establishment was affected little, with establishment generally high or low depending on the population under study, and independent of female ovipositional preference.

After factors affecting establishment of larvae, the major mortality factor was parasitism, including death caused by inquilines, so the probability of survival to the final larval stage which emerges from the gall integrates the major effects of aborted and parasitized galls (Figure 4). Probability of survival is therefore the final test of a preference-performance linkage. The evidence again suggests poor linkage of female preference for shoot length and performance (Figure 6, Table II). The one positive and significant relationship was in the Flagstaff population, the two *P. pustulator* populations showed almost flat responses, and the Sapporo population showed a strongly negative slope, but was not statistically significant. The response in the Sapporo population resulted from a statistically significant increase in parasitism as shoot length increased ($r^2 = 0.95, p < 0.001$).

If females respond to a variable in plant quality not captured in the synoptic index of shoot length, then we may find improved correlations, if shoot length is excluded, between probability of attack and probability of larval establishment, or larval survival (Stein & Price, 1995). In fact, in seven cases treated in Table II, r^2 values changed
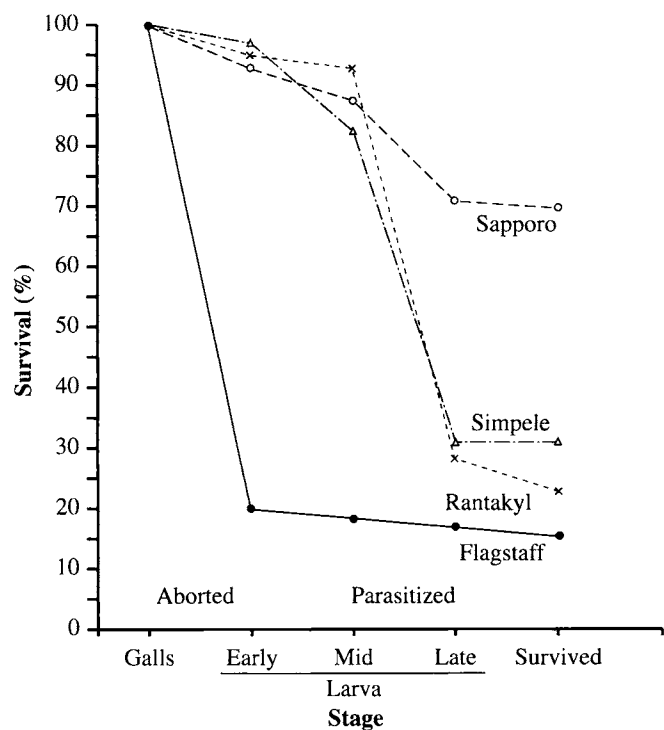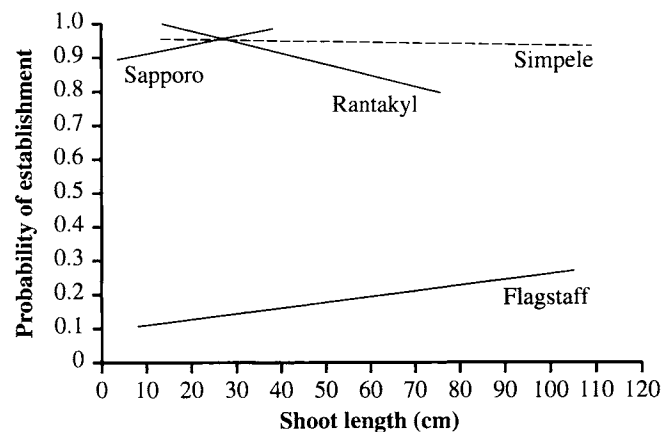

FIGURE 5. Probability of a larva establishing a feeding site per gall in relation to shoot length in the four populations of *Pontania* studied. Regression equations are provided in Table II.

less than 0.09 or accounted for ± 9% of the variance explained by shoot length. The significance of the relationship did not change in these seven cases when compared to the regressions using shoot length given in Table II. In the one case of a larger change in r^2 (r^2 reduced from 0.51 to 0.32) for the Rantakylä population and probability of establishment of larvae in galls, the relationship between probability of attack and larval establishment was not significant. Thus, using probability of attack by females provides no significant increase in the explanatory variables concerning larval establishment and survival.
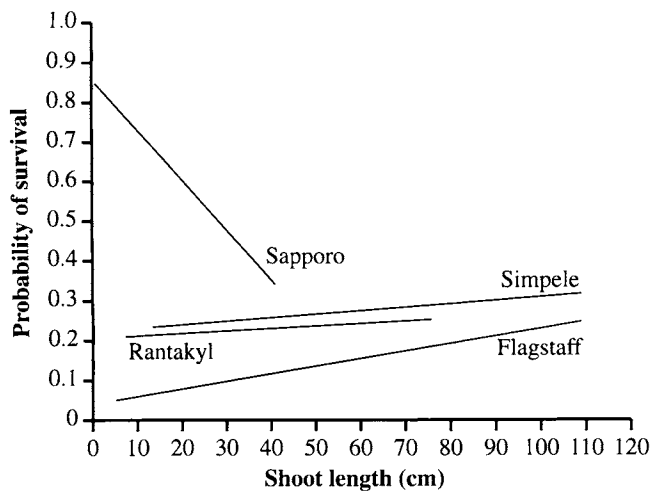
FIGURE 6. Probability of a larva surviving to completion of feeding per gall in relation to shoot length in the four populations of *Pontania* studied. Regression equations are provided in Table II.

Discussion

Before this study, only one *Pontania* species had been investigated for linkage between ovipositional choices and larval survival, and the results differed with different investigators and at different densities. Therefore, adding another three *Pontania* species in order to evaluate the Phylogenetic Constraints Hypothesis significantly broadens our comparative power within the genus *Pontania* and across the related gall-inducing genera of sawflies, *Euura* and *Phyllocolpa*.

Concerning the Phylogenetic Constraints Hypothesis, we have now established that there is broad conformity across all genera for strong preference for long shoots during oviposition. However, this pattern may be lost at high densities in the only species studied to date that reaches high population levels: *Pontania* near *pacifica* (Clancy, Price & Sacchi, 1993). Such high preference for long rare shoots on young ramets results in resources that are patchy, with patches lasting perhaps only a few years before ramets age beyond a utilizable state (Figure 2). Thus, the component of the hypothesis concerning ovipositional preference is supported by the present study and the consequences for the population dynamics of the *Pontania* species appear to be closely similar to those for *Euura* on which the hypothesis was developed.

However, the linkage between ovipositional preference and larval performance in *Pontania* was very weak or absent, counter to the Phylogenetic Constraints Hypothesis. We cannot understand the selective pressures and adaptive responses acting in populations by glimpses involving one generation, especially if species do not conform to the existing hypothetical framework we use to investigate them. Therefore, having found three *Pontania* species with weak or absent correlations between ovipositional preference for longer shoots and larval performance, we should consider the validity of the original hypothesis and possible alternative hypotheses. We discuss four mechanisms that may negate discovery of the predicted patterns.

The first hypothesis, which we favor, is that preference is so strongly developed in relation to performance that we cannot detect any strong trends in the linkage. Each species of *Pontania* utilizes only one species of host plant showing much more specificity than many species studied for preference-performance linkages (Thompson, 1988; Thompson & Pellmyr, 1991). These sawflies also focus attacks on a very narrow range of plant ages within the host plant species, when growth is very rapid on young plants and before ontogenetic and physiological aging becomes important (Roininen, Price & Tahvanainen, 1993). Thus, the adaptive shift to high preference-performance linkage is more or less a fixed trait. In general, then, females would select the best possible resources for gall formation in which performance is as high as possible, given the state of resources in any one year. To test this hypothesis it would be valuable to experimentally extend the range of ages attacked by females and to evaluate the behavioral responses of the females themselves and the survival of any progeny produced. We have performed such experiments on the stem galler, *Euura lasiolepis*, and have shown that on low-preference shoots, eggs are frequently not laid in galls and any progeny have a high probability of death before maturity (Preszler and Price, 1988; Price, Craig & Roininen, 1995).

If this scenario is correct, then the Plant Vigor Hypothesis (Price, 1991) is supported, which argues that many herbivores attack the most vigorous modules in a plant population, rather than modules on stressed plants, because of various beneficial qualities of rapidly growing modules. These would include rapid growth itself, large leaves, high concentrations of certain phytochemicals, and so on.

A second hypothesis suggests that larval performance is so unpredictable that a population of sawflies is unlikely to evolve behavioral traits in females relevant to the probability of larval survival. For example, high mortality caused by parasitoids and inquilines is not a variable that female behavior can readily evolve in response to, and such mortality could negate any adaptive shift that maximizes a preference-performance linkage. This hypothesis gains credence from the wide range of mortality factors and levels of mortality seen in the survivorship curves in four populations (Figure 4). If such variation occurred within species from generation to generation, then selection for female preference is likely to be weak. Noteworthy also is the evidence that the only species of *Pontania* that showed a significant preference-performance linkage was on *S. scouleriana* in which abortion, a plant resistance trait, was high and parasitism was very low (Figure 4). However, this hypothesis cannot account for the high preference for oviposition into long shoots observed in all three species.

A third hypothesis argues that high preference, as seen in the three species studied, is selected for independently of larval performance. It is adaptive for females to oviposit into rapidly growing shoots, perhaps, because of softer tissue, quicker and easier penetration and less wear on the ovipositor. Also, the shoot is a stronger sink for resources which can be tapped by the developing gall, vigorous meristems may be more easily diverted in their development to the formation of gall tissue, and long shoots may have higher levels of the oviposition stimulant, as in the

case of the stem gallers, *Euura lasiolepis* (Roininen *et al.*, 1998) and *E. amerinae* (Kolehmainen *et al.*, 1994). This hypothesis suggests a role of plant quality variation that reinforces the mechanisms invoked in our first hypothesis. It also provides a reason, as in the second hypothesis, that ovipositional preference can exist without a corresponding larval performance.

The fourth hypothesis suggests that gall formation on the leaf lamina is less constrained than that on stems, petioles, leaf midribs, and buds utilized by *Euura* species. The leaf is a source of photosynthate and a sink for mineral nutrients, probably with relative uniformity in leaf lamina nutrient status, especially during gall formation early in leaf development. Clancy, Price & Sacchi (1993) found large galls on small leaves, suggesting that leaf size was not correlated with limiting resources for gall formation. We may well find with further study that *Pontania* species forming large galls, such as the three species in the present study, will show strong preference for longer shoots, whereas those inducing smaller galls, such as *Pontania* near *pacifica*, are less constrained in their choices. In contrast to the leaf lamina, the modules used by *Euura* species produce little or no photosynthate, consisting mainly of vascular tissue (except for buds), and show extreme variation in lengths, diameters, and developmental rates, creating a much more heterogeneous array of potential resources for *Euura* species. For the genus *Phyllocolpa*, leaf length is important because it defines the possible length of the gall and the resource supply for the larva. This constraint promotes the development of a strong preference for longer shoot-length classes, and a preference-performance linkage was found for one species (Price, Clancy & Roininen, 1994; Price & Ohgushi, 1995).

These four hypotheses could be tested by longer term studies on each species on a larger number of populations, and with the experimental approaches mentioned above. However, finding additional populations of the species in this study to work on is exceedingly difficult, so we have had to be opportunistic. The fact that the species in Arizona and on Hokkaido are unrecorded in major catalogues suggests that they have been rare for a long time (Smith, 1979; Yukawa & Masuda, 1996). In addition, populations of all species are small, making large sample sizes on a wide range of plant ages and module lengths difficult to achieve.

An interesting case of apparently changing preference-performance linkage is evident in two studies on *Pontania* sp. near *P. pacifica* in Flagstaff. When populations were very high between 1981 and 1986, there was no evidence of a preference for longer leaves that occur on longer shoots, and performance was not correlated positively with leaf size (Clancy, Price & Sacchi, 1993). Mortality caused by parasitoids and inquilines was high. Subsequently, the population declined dramatically and was studied in 1989 by Stein & Price (1995), who found strong preference-performance linkage and relatively low levels of mortality caused by natural enemies. Unfortunately, the methods used in the two studies were not identical, but the results suggest the possibility that preference-performance relationships change as populations flush and crash, and that long-term studies on one species would be enlightening.

Certainly, the study of these apparently rare species of *Pontania* reveals that the rarity of resources is a prime contributing factor. Disturbance in the managed landscapes we examined is infrequent and when it occurs it may obliterate young willow populations. For example, the site near Sapporo was created by landfill, but it is now cleared for construction of a golf course, and each population of young willows inevitably ages if undisturbed, rendering it unacceptable to *Pontania* sawflies. This highly dynamic state of resource-driven population change is well documented for the stem-galling sawfly, *Euura amerinae* L., which can only persist in a population of young willows for about seven years (Roininen, Price & Tahvanainen, 1993). Any form of strong preference for rare modules like young plants or long shoots or both is likely to render a species into the category of a rare species that is patchily distributed and with population dynamics and distribution dictated by disturbance over a landscape. Even though populations may be small and opportunities brief, we argue that more investigations on these kinds of species will advance our understanding of insect herbivore preference-performance linkages, the reasons for rarity, and patterns in insect population dynamics.

Our comparative approach to understanding the distribution and abundance of gall-inducing sawflies has advanced considerably since the last overview published in 1994 (Price, Clancy & Roininen, 1994). We have tripled the number of *Pontania* species studied, and added a study on the preference-performance linkage of a *Phyllocolpa* species to the records on preference of three congeneric species. Within the group of gall-inducing sawflies, we now have preference-performance studies on seven *Euura* species, four *Pontania* species and one *Phyllocolpa* species (Price, Clancy & Roininen, 1994; Price, Craig & Hunter, 1998). The species represent the full geographical range of the group in the holarctic realm. Concerning the Phylogenetic Constraints Hypothesis, the *Euura* species are fully consistent with the predictions, *Phyllocolpa* species need more study, but are generally consistent, and *Pontania* species are mostly consistent in terms of oviposition preference, and predicted distributions and abundances, but the factors limiting the detection or presence of a preference-performance linkage are in need of much more detailed investigation. New developments on the systematics of the genus *Pontania* will afford us a finer comparative approach to species within the genus (Zinovjev, 1998). However, at this time, the genus *Pontania* provides a most heuristic set of examples and opportunities for testing the Phylogenetic Constraints Hypothesis, probably necessitating further modifications, refinements, or extensions.

Acknowledgements

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