Biology of Hybridization between *Farfugium japonicum* and *F.hiberniflorum* (Compositae)

Tetsukazu YAHARA
The Botanical Gardens, Faculty of Science, University of Tokyo. 3-7-1
Hakusan, Bunkyo-ku, Tokyo 112, Japan

Hirofumi YAMAGUCHI
Department of Genetics, Facutly of Agricuture, Osaka Prefectural University.
4 Mozu-Ume-machi, Sakai 591, Japan

Takakazu YUMOTO
Laboratoly for Plant Ecological Studies, Faculty of Science, Kyoto University.
Sakyo-ku, Kyoto 606, Japan

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Introduction

Although the level of interspecific hybridization is generally limited by various isolating mechanisms that ensure the coexistence of two closely related species, interspecific hybrids and hybrid derivatives are not rare in the field. Thus interspecific hybridization has been of continued interest in plant biosystematics up till today. There have been numerous studies on this subject, and the genetic background of hybrid sterility, the process of the introgression or the stabilization of hybrid derivatives, and the role of hybridization in evolution have been well documented with reference to generalizations about the isolation mechanisms between species and the process of plant speciation (Levin, 1978, 1979).

Compared with our relatively rich knowledges on these aspects of hybridization, relatively few efforts have been made to analyze in quantitative terms the process of interspecific pollination, which causes the production of hybrid seeds. In entomophilous plants, the behaviour of pollinators affects the frequency of hybridization (Levin, 1972). It has been demonstrated that pollen dispersal by insects is largely restricted within rather narrow areas (Levin and Kerster, 1974; Handel, 1983), and it is expected that the degree of spatial isolation has a profound effect on limiting the level of hybridization. The first attempt to analyze the effect of spatial isolation on the frequency of interspecific pollination was made by Levin (1972) by utilizing the difference of pollen size between two partially sympatric species of *Phlox*. He demonstrated that the level of interspecific pollen exchange drops abruptly in contiguous populations compared with sympatric populations, and that it continues to decline in a roughly exponential fashion as the distance between populations of two species increases. However, his paper did not include quantitative data about the incidence of conspecific and interspecific pollinator flights, or on the effect of the density and the relative frequency of parental species upon the level of interspecific pollen exchange, even though he referred to the importance of these factors. In the present paper, the result of an attempt to quantify these factors relating to the level of hybridization is presented in order to evaluate the roles of spatial isolation and pollinator behaviour in limiting the interspecific hybridization in flowering plants more exactly.

The Plants

Farfugium Lindl. is a small genus of Compositae, consisting of only two species: *F. japonicum* (L.f.) Kitamura and *F. hiberniflorum* (Makino) Kitamura. It belongs to the tribe Senecioneae and is close to *Ligularia* (Koyama, 1968). Both species are perennial herbs with short and thick rhizomes and radical evergreen leaves. Scapes without leaves appear in October to November and grow to a height of 0.5–1.2 m. Heads are bright yellow and arranged in compound corymbose inflorescences.

F. japonicum is widely distributed in the western part of the Japan mainland, the Okinawa Islands, Taiwan, the eastern coast of the continental China and the southern coast of Korea. It grows usually in open places near seacoasts. *F. hiberniflorum* is endemic to Yakushima and Tanegashima Islands and abundant especially on Yakushima Island. The highest peak on Yakushima is 1935 m above sea level, and *F. hiberniflorum* grows in shady and wet places near streams from sea level to about 1000 m elevation. In the lowland of Yakushima, *F. japonicum* is abundant in open and semi-shady places along roadsides, forest margins or stream margins, and is frequently sympatric with *F. hiberniflorum*. Sympatric occurrences of these species are, in most cases, induced by human activities such as construction of roads and railways for forestry. In mixed populations, hybrids are commonly found.

Two species share the same chromosome number $2n=60$ (Arano, 1962; Koyama, 1968). Both are self-incompatible. Hybrids show a very low level of seed fertility (about 1–2%), and backcrossed generation is doubtful. *F. japonicum* and *F. hiberniflorum* are distinct from each other in such morphological features as leaf shape, depth and shape of serration, leaf texture, leaf color, size of head, color of pappus, hairiness of scape, and others, and it is easy to discriminate hybrids which show intermediate morphology from the two parental species (Fig. 1).

Results and Discussion

In order to examine the effects of the density and relative frequency of parental species upon the frequency of hybrids, a line transect was put in a mixed population along the railway for forestry at 140 m above sea level and ca. 4 km N from Ambo. Numbers of individuals of parental species and of hybrids were counted in each 5 m block. Along this transect, *F. hiberniflorum* is distributed rather continuously, whereas *F. japonicum* has a patchy distribution (Fig. 2). The frequency of *F. hiberniflorum* and that of *F. japonicum* do not show any correlation. On the other hand, the hybrids exhibited patchy dis-

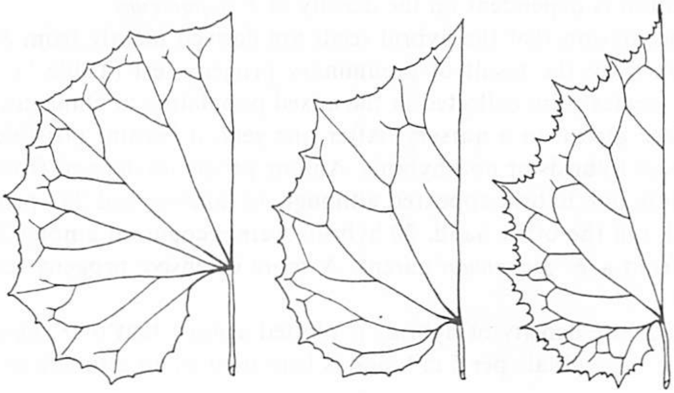

Fig. 1. Leaves of *Farfugium*. Left: *F. japonicum*. Center: Hybrid. Right: *F. hiberniflorum*. 1/4.

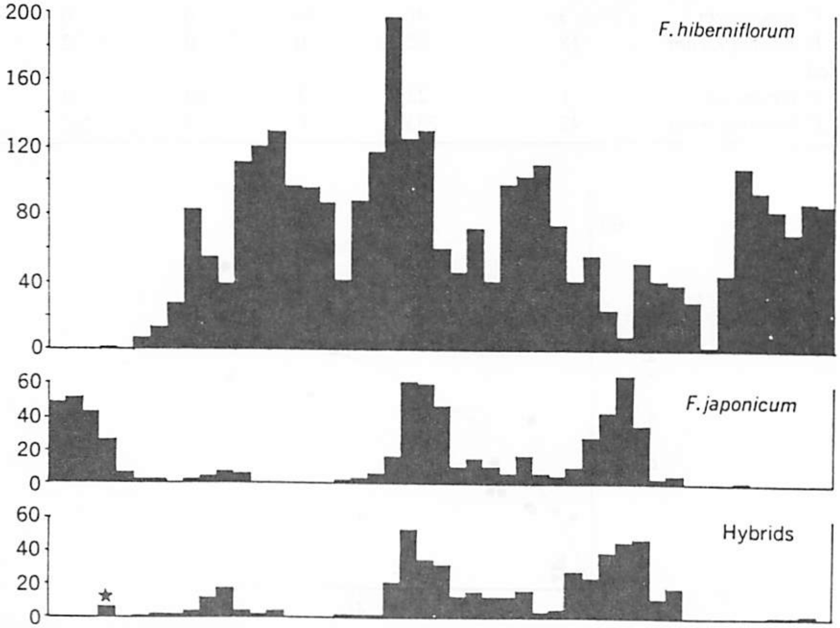

Fig. 2. Distribution of *Farfugium hiberniflorum*, *F. japonicum*, and hybrids along the transect. Histogram indicates number of individuals in each 5 m block.

tribution, and their distribution overlaps very well with that of *F. japonicum*. This pattern implies that 1) hybrids are mainly derived from seeds of *F. japonicum*, 2) dispersal distance of hybrid seeds is restricted, and 3) the frequency of hybridization is dependent on the density of *F. japonicum*.

The speculation that the hybrid seeds are derived mainly from *F. japonicum* is supported by the result of preliminary progeny test (Table 1). Achenes of parental species were collected in the mixed population at Onnagawa, and progenies were grown in a nursery. After one year, it became possible to identify progenies as hybrids or non-hybrids. Among progenies derived from *F. hiberniflorum*, only one hybrid appeared although 42 families and 233 progenies were examined. On the other hand, 20 hybrids were recognized among 22 progenies derived from a *F. japonicum* parent. A more extensive progeny test is now in progress.

In Fig. 3, the density of hybrids is plotted against that of *F. japonicum*. The number of individuals per 5 m block is here used as an estimate of density. At

Table 1. Frequencies of putative hybrids in the progenies grown from seeds collected in pure and mixed populations.

	No. families	No. plants	*F. japonicum*	Hybrid	*F. hiberniflorum*
Pure					
F. japonicum	3	46	46	0	0
F. hiberniflorum	18	72	0	0	72
Mixed					
F. japonicum	1	22	2	20	0
F. hiberniflorum	42	233	0	1	232

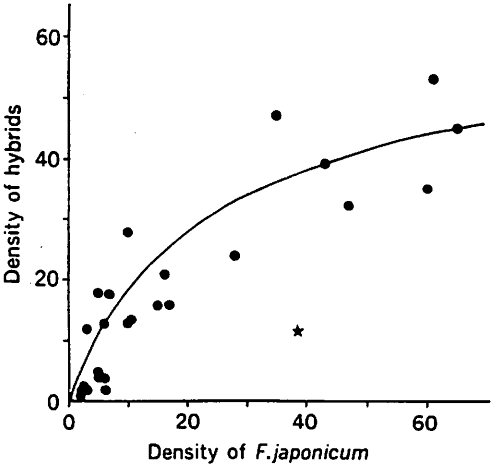

Fig. 3. Relationship between the density of *Farfugium japonicum* and that of the hybrid.

low densities of *F. japonicum*, the density of hybrids increases nearly proportionally to that of *F. japonicum*. This result implies that the pre-crossing isolation mechanism is not effective in preventing interspecific hybridization when *F. japonicum* is present at low densities. At high densities of *F. japonicum*, the density of hybrids increases gradually and some factors seem to operate in decreasing the rate of hybridization and increasing the rate of crossing within *F. japonicum*.

Another notable point in Fig. 3 is that one block designated by an asterisk shows a relatively low density of hybrids inspite of its high density of *F. japonicum*. This block is located at the edge of the continuous distribution of *F. hiberniflorum* (an asterisk in Fig. 2), and the relatively low level of hybrid density might be attributed to limited pollen sources of *F. hiberniflorum*.

It is expected that pollinator behaviour plays an important role in shaping the patterns shown in Figs. 2 and 3. *F. japonicum* is pollinated by bees, house flies, syrphids and butterflies when it grows, as it usually does, in open places. In contrast, *F. hiberniflorum* usually grows in shady places, where flies and butterflies are neither frequent nor active; this species is pollinated primarily by a solitary bee, *Colletes perforator* Smith. This bee has pollen baskets on its hindlegs, forages actively for pollen from October to December on Yakushima, and frequently visits both *F. japonica* and *F. hiberniflorum*.

Figure 4 shows the flight pattern of *Colletes perforator* observed on 1 and 2 December 1985 in the area between 10 and 30 m from the origin of the transect. The population is subdivided into two subpopulations kept 2 m apart by the forestry railway. As is clearly shown in Fig. 5, *Colletes* seldom moves between two subpopulations even if they are isolated only by a short distance. This flight pattern apparently results from the habit of *Collectes* of flying between near-neighbour plants at the same height on successive visits. The subpopulation on the upper side of Fig. 4 is located on a steep slope made by railway construction. The more or less horizontal flight pattern suggests that *Colletes* tends to fly nearly at the same height. Similar habits have been demonstrated clearly in the honeybee (Faulkner, 1976; Levin and Kerster, 1973).

Figure 5 shows the distribution of flight distances of *Colletes* between successively visited inflorescences of *Farfugium*; it shows an L shape of the sort that has been well established for many kinds of pollinators (*cf.* Levin and Kerster, 1968, 1969; Pyke, 1978, 1981; Waddington, 1983).

Observed numbers of conspecific and heterospecific flights are compared with expectations under the assumption of random or nearest-neighbour foraging in Table 2. The observed numbers of conspecific flights significantly exceed the number expected under an assumption of random flight, and fall significantly below that number under an assumption of flight between nearest neighbours. Thus near-neighbour foraging results in frequent interspecific pollen exchange.

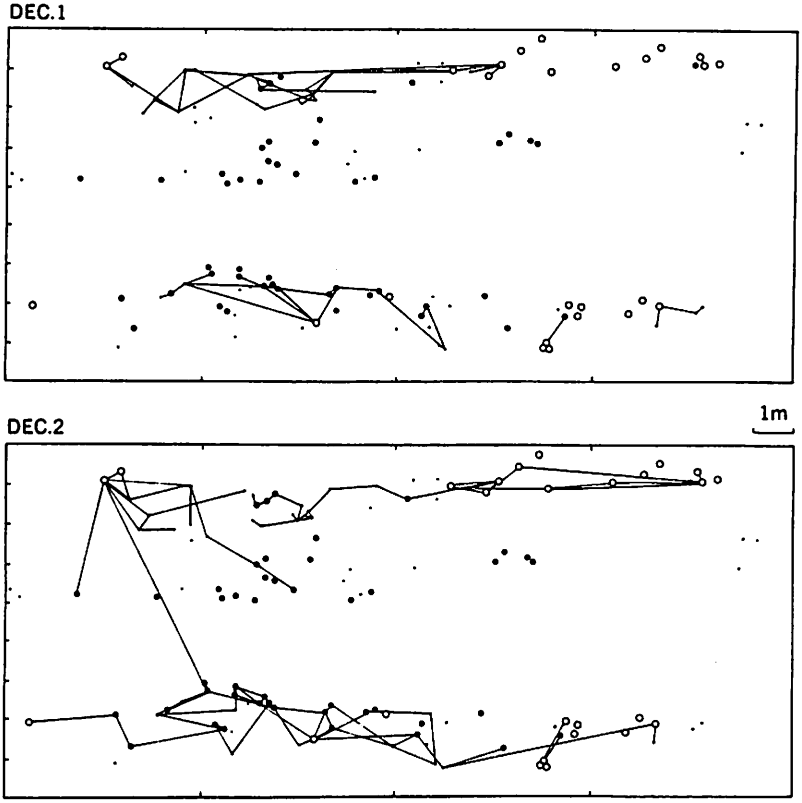

Fig. 4. Flight pattern of *Colletes perforator*. Open circle: flowering scape of *F. hiberniflorum*. Solid circle: flowering scape of *F. japonicum*. Dot: flowering scape of the hybrid.

Table 2. Observed and expected frequencies of nine kinds of successive flights in the mixed population. HIB: *Farfugium hiberniflorum*. JAP: *F. japonicum*. HYB: Hhybrid.

Successive	Observed	Random Foraging			Nearest-Neighbour Foraging		
Flight	No.	Expectation	E–O		Expectation	E–O	
JAP-JAP	32	21.7	+10.3	*	44.9	−12.9	*
JAP-HYB	18	26.9	−8.9		14.9	+3.1	
JAP-HIB	11	20.3	−9.3		1.2	+9.8	
HYB-HYB	49	27.5	+21.5	*	54.3	−5.3	
HYB-JAP	16	33.1	−17.1		15.7	+0.3	
HYB-HIB	11	15.4	−4.4		6.0	+5.0	
HIB-HIB	28	17.7	+10.3	*	37.9	−9.9	*
HIB-JAP	8	9.7	−1.7		4.8	+3.2	
HIB-HYB	13	21.6	−8.6		6.3	+6.7	

* Statistically significant ($X^2 < P = 0.005$).

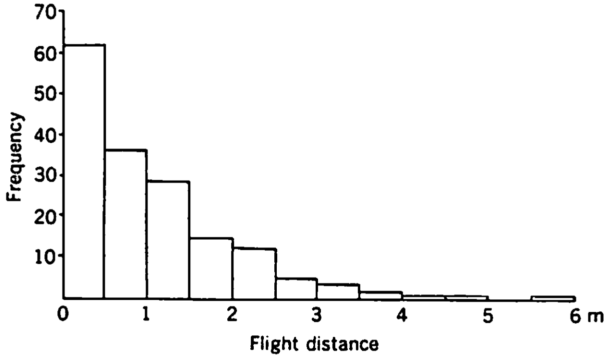

Fig. 5. Distribution of flight distances of *Colletes perforator*.

An important point that should be considered here is the effect of the spatial distribution of parental species upon the degree of interspecific flight. Under a given flight pattern, the relative frequency of conspecific or interspecific flight is a function of the degree of aggregation of each species and the distribution correlation between two species. Figure 6 shows the measurements of these two factors in the pollinator observed site. The degree of aggregation was measured

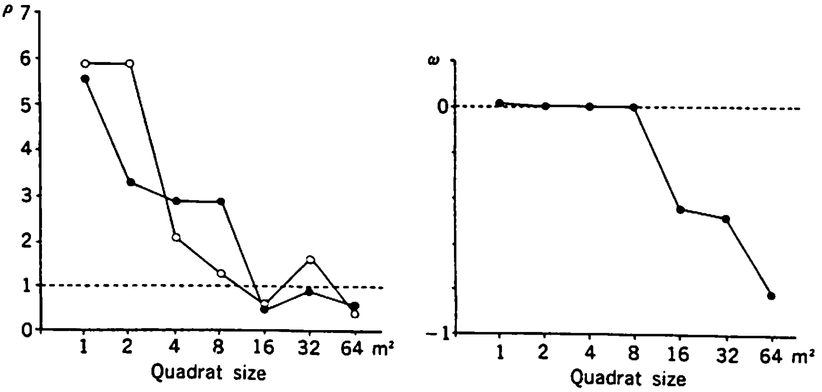

Fig. 6. Left: Rho index-quadrat size relations in *Farfugium hiberniflorum* (open circle) and *F. japonicum* (solid circle). Right: Omega index-quadrat size relations between *F. hiberniflorum* and *F. japonicum*. Rho and omega indices are calculated based on the distribution of flowering scapes shown in Fig. 4.

by the rho index and the distribution correlation was measured by the omega index, both of which were devised by Iwao (1972, 1977), based on the regression pattern between mean density and mean crowding per quadrat. The rho value is equal to or larger than unity in random and aggregated distributions respectively. If there is some colonial structure, the rho values change between different-sized quadrats, and the turning point of the rho-quadrat size plot indicates the quadrat size approximately equal to the clump area.

The rho values of *F. hiberniflorum* and *F. japonicum* decrease as quadrat size increases. Both species are considered to have loose colonies, the size of which is estimated approximately as 2 m^2 in *F. hiberniflorum* and 8 m^2 in *F. japonicum*.

The omega value is zero if two species are randomly distributed, -1 if completely exclusive, and unity if completely correlated. In quadrats smaller than 8 m^2, the omega values are approximately zero and the association of two species is regarded as nearly random. In the quadrats larger than 16 m^2, the omega values between two species are negative and the distribution of two species is exclusive. The larger the quadrat size is, the smaller the omega value is and the more exclusive the distribution of two species is. It is suggested that the environmental heterogeneity that causes the exclusive distribution of two species increases with an increase in quadrat size.

These spatial distribution patterns explain well the excess of conspecific flight compared with the expectation under the assumption of random foraging. The interaction of the near-neighbour foraging of the pollinator and the spatial aggregation and exclusive distribution of the parental species will restrict the frequency of hybridization below some level. The saturation of hybrid-increase shown in Fig. 3 may be attributed to this interaction. In blocks with a low density of *F. japonicum*, the plants of this species were observed to be less aggregated. Under these circumstances, the frequency of hybridization is expected to increase proportionally to increasing density of *F. japonicum* if the pollen source of *F. hiberniflorum* is not limited. This prediction agrees well with the result shown in Fig. 3. Although the frequency and distribution of hybrids along the transect may be formed under compound interractions between various factors, their primary determinants are suggested to be the density and spatial distribution of the parental species and the foraging behaviour of the pollinator.

As was demonstrated clearly by Levin and Kerster (1974), pollen and seed dispersals are in general very restricted in flowering plants. The results of this study agree with their conclusion. Levin (1972, 1978) emphasized the effectiveness of spatial isolation as a natural consequence of the restriction of gene flow in the field. Levin (1972) analyzed the effect of the proximity of heterospecific populations on limiting the level of pollen exchange between two species of *Phlox*. The circumstances of *Phlox* studied by him are different from those of *Farfugium* in some respects. In *Phlox*, the interspecific pollen exchange

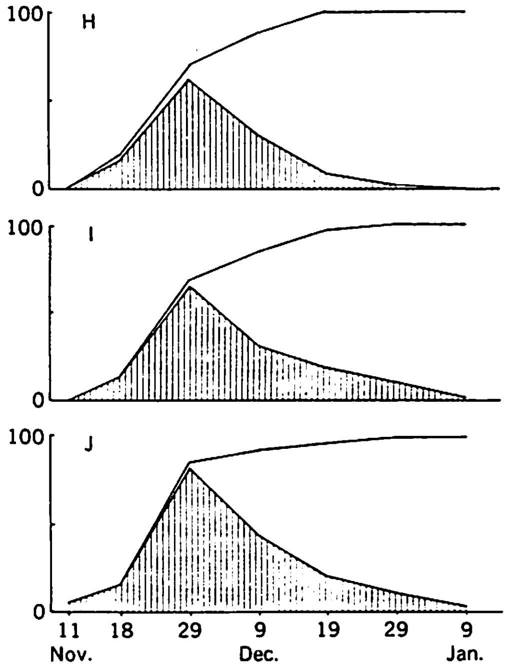

Fig. 7. Flowering phenologies of *Farfugium hiberniflorum* (H), *F. japonicum* (J), and the hybrid (I). Percentage (inner line) and cumulative percentage (outer line) of heads in anthesis on a given day are shown.

was relatively restricted even in the absence of spatial barriers; no hybrids were detected in the field; the floral morphology of the two species was conspicuously different; and pollinator constancy may play an important role in limiting the level of interspecific pollination. On the other hand, in *Farfugium*, interspecific pollinator flights are not so restricted; hybrids are rather frequent in mixed populations; and there is no obvious difference in floral morphology. It is notable in relation to the present discussion that two species of *Farfugium* share one primary pollinator species, and their flowering periods are largely overlapping (Fig. 7). *Farfugium* flowers from late autumn to early winter, in which season both the diversity of pollinator fauna and the density of pollinators are low. Seed fertility in two species of *Farfugium* shows a wide range of variation, from less than 10% to nearly 100%, and pollinator limitation of seed set is probable in many individuals (Yamaguchi *et al.*, unpublished). Under these circumstances, pollinator sharing rather than specialization seems to be adaptive in *Farfugium*. The overlap of flowering periods may support this hypothesis.

Relatively frequent interspecific flight is regarded as a result of pollinator sharing.

Except for these differences, the result of the present study agrees well with Levin's (1972) view that spatial isolation limits the level of hybridization under the interaction with pollinator foraging behaviour. Since flowering plants are sedentary, this interaction appears to be one of the primary factors operating in the isolation between entomophilous plant species.

Summary

The hybridization between *Farfugium japonicum* and *F. hiberniflorum* was quantitatively examined with special reference to pollinator foraging behaviour. Pollinator flights between two species were frequently observed in mixed populations, but the observed frequency of conspecific flight exceeded expectations under the assumption of random flight. This excess is well explained by the interaction of the near-neighbour foraging of the pollinator and the spatial aggregation and exclusive distribution of two *Farfugium* species. As expected from observed interspecific flight of the pollinator, progenies derived from achenes of *F. japonicum* collected in a mixed population included hybrids at high frequency. The density of hybrids in the field increased nearly proportionally to that of *F. japonicum* at low densities of *F. japonicum*, and increased gradually at high densities of *F. japonicum*. It is suggested that the exclusive distribution of two species at high densities operates to decrease the rate of hybridization under interaction with the near-neighbour foraging of the pollinator.

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Literature Cited

Arano, H. (1962). Cytological studies in subfamily Carduoideae (Compositae) of Japan. VIII. The karyotype analysis in tribe Senecioneae. *Bot. Mag. Tokyo*, **75**: 401–410. In Japanese with English summary.

Faulkner, G. J. (1976). Honeybee behaviour as affected by plant height and general color in Brussels sprouts. *Journ. Apic. Res.*, **15**: 15–18.

Handel, S. N. (1983). Pollination ecology, plant population structure, and gene flow. *In* Real, L. (ed.), Pollination Biology, pp. 163–211. Academic Press.

Iwao, S. (1972). Application of the $\overset{*}{m}$-m method to the analysis of spatial patterns by changing the quadrat size. *Res. Popul. Ecol.*, **14**: 97–128.

Iwao, S. (1977). Analysis of spatial association between two species based on the interspecies mean crowding. *Res. Popul. Ecol.*, **18**: 243–260.

Koyama, H. (1968). Taxonomic studies on the tribe Senecioneae of Eastern Asia. II. Enumeration of the species of Eastern Asia. *Mem. Fac. Sci. Kyoto Univ. Ser. Biol.*, **2**: 19–60.

Levin, D. A. (1972). Pollen exchange as a function of species proximity in *Phlox*. *Evolution*, **26**: 251–258.

Levin, D. A. (1978). The origin of isolation mechanisms in flowering plants. *Evol. Biol.*, **11**: 185–317.

Levin, D. A. (ed.) (1979). Hybridization. Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania.

Levin, D.A. and H.W. Kerster (1968). Local gene dispersal in *Phlox*. *Evolution*, **22**: 130–139.

Levin, D.A. and H.W. Kerster (1969). The dependence of bee-mediated pollen and gene dispersal upon plant density. *Evolution*, **23**: 560–571.

Levin, D.A. and H.W. Kerster (1973). Assortative pollination for stature in *Lythrum salicaria*. *Evolution*, **27**: 144–152.

Levin, D.A. and H.W. Kerster (1974). Gene flow in seed plants. *Evol. Biol.*, **7**: 139–220.

Pyke, G. H. (1978). Optimal foraging: Movement patterns of bumblebees between inflorescences. *Theor. Popul. Biol.*, **13**: 72–98.

Pyke, G. H. (1981). Optimal foraging in hummingbirds: Rule of movement between inflorescences. *Anim. Behav.*, **29**: 889–896.

Waddington, K. D. (1983). Foraging behavior of pollinators. *In* Real, L. (ed.), Pollination Biology, pp. 213–239. Academic Press.