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POLLEN-MEDIATED GENE FLOW IN CUCURBITA FOETIDISSIMA (CUCURBITACEAE)[1]

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Pollen-mediated gene flow along a linear series of patches of the functionally gynodioecious, bee-pollinated *Cucurbita foetidissima* was assessed using electrophoretic analysis of seed allozymes. Gene flow was documented at distances up to 0.7 km. For the 13 patches examined, interpatch pollen must have sired from 0% to 48.3% ($\bar{X}$ = 8.5%) of the seeds of monoecious plants (hermaphrodites). Rates of interpatch siring of seeds of pistillate plants (females) averaged 20.4% (range 8.6%–40%) for the three patches examined. Heterogeneity among fruits in seeds sired by interpatch pollen indicates that the arrival of interpatch pollen is clumped with respect to stigmas. Within patches, plants of the same sex type usually shared identical five-locus genotypes, suggesting that clonal propagation predominates. Since approximately 90% of seeds are sired by intrapatch pollen, seeds of monoecious plants appear to result primarily from geitonogamous (self-) fertilization. This may help explain the existence of female plants in natural populations, since self-fertilization has been shown to severely reduce the survival of seedlings in this species.

The rate and distance of gene flow, together with population size and the intensity of selection, determine the spatial scale of evolutionary processes (Wright, 1943, 1968). Thus the measurement of gene flow is of major importance in evolutionary biology (Levin and Kerster, 1974; Ellstrand and Marshall, 1985; Slatkin, 1985a). An important component of total gene flow in many plant populations may be achieved through the movement of pollen. Since the rate of gene flow by pollen is likely to be strongly affected by the spatial structure and density of plant populations (Levin and Kerster, 1974; Handel, 1983), community composition (Campbell, 1985), pollinator identity (Schmitt, 1980; Waser, 1982, 1983, 1988; Waddington, 1983), and floral traits (Waser and Price, 1984; Tonsor, 1985; Campbell and Waser, 1989), only after measurement of gene flow by pollen in a large number of species will generalities, if any, emerge (Ellstrand and Marshall, 1985; Ellstrand, Devlin, and Marshall 1989).

Levin (1984) characterized our knowledge of gene flow in plant populations as "an exercise in the subjunctive" due to the lack of direct measures of gene flow in plant populations. Documenting gene flow due to pollen dispersal has proven difficult. The rate and distance of pollen-mediated gene flow has been estimated indirectly using the distances flown by pollinators, the distance traveled by marked pollen or pollen mimicking dyes, or the distance from the paternal parent that marker genes can be found among seeds of synthetic plant populations (reviewed by Handel, 1983; see also Murawski and Gilbert, 1986; Ordway et al., 1987; Smyth and Hamrick, 1987; Campbell and Waser, 1989; Olesen and Warncke, 1989). Most of these studies have been aimed at measuring gene flow within patches, while gene flow among patches has been ignored or assumed to be infrequent. Recently, electrophoretic analysis of seed or seedling allozymes has been used to obtain direct measures of intra- and interpatch pollen-mediated gene flow in natural plant populations (Ellstrand and Marshall, 1985; Meagher, 1986; Kirkpatrick and Wilson, 1988; Ellstrand, Devlin, and Marshall, 1989; Devlin and Ellstrand, 1990). Here we use paternity exclusion based on allozyme variation to measure pollen-mediated interpatch gene flow in buffalo gourd, *Cucurbita foetidissima.*

Cucurbita foetidissima HBK (Cucurbitaceae), is a long-lived, xerophytic, perennial native to deserts and grasslands of the western United States and northern Mexico. Like all members of its genus, buffalo gourd often inhabits sites of natural or human disturbance, chiefly roadsides in the area of this study. Plants annually produce from one to 30 deciduous stems, each up to 12 m long, and can propagate vegetatively by producing adventitious roots at the leaf nodes. Rooted nodes may become autonomous when vines die back each fall. Because of their association with roadways, populations often consist of a linear series of patches separated from one another by tens to thousands of meters. Patches contain from one to a few hundred plants (ramets), but usually less than 20 in the area of this study.

Populations of *C. foetidissima* are functionally gynodioecious, containing both hermaphroditic (monoecious) and female (gynoecious) plants (Dossey, Bemis, and Scheerens, 1981; Kohn, 1989). Hermaphroditic plants produce separate staminate and pistillate flowers, while female plants produce only pistillate flowers. Plants do not change sex (J. R. Kohn, personal observation), and gender appears to be controlled by a single locus (Yousef, 1976; Dossey, Bemis, and Scheerens, 1981; J. R. Kohn, unpublished data). The large, yellow, bell-shaped flowers last a single morning and resemble those of cultivated squashes. Principal pollinators in the area of this study are four species of specialist Anthophorid bees called squash bees: *Peponapis pruinosa, P. utahensis, Xenoglossa angustior,* and *X. strenua* (Hurd and Linsley, 1964; J. R. Kohn, personal observation). Other visitors include bum-

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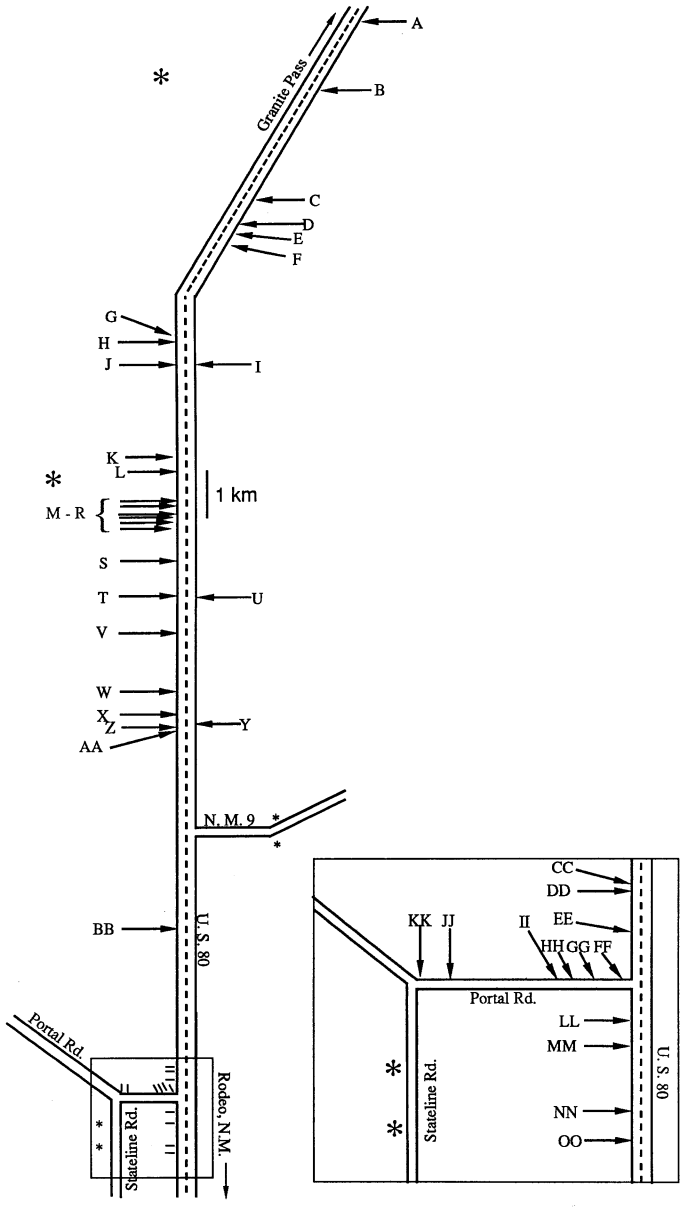

Fig. 1. Location of all patches of *Cucurbita foetidissima* in the study area along U.S. Hwy 80 in southwestern New Mexico. Asterisks mark locations of nearby patches.

blebees (*Bombus* spp.), honeybees *Apis mellifera,* and sweat bees (*Agapostemon* spp.). However, squash bees are by far the most abundant visitors, forage earlier in the morning, and are the most efficient (sensu Primack and Silander, 1975) pollinators of buffalo gourd (S. L. Buchmann, unpublished data) and other cucurbits (Hurd, Linsley, and Whitaker, 1971; Hurd, Linsley, and Michelbacher, 1974; Tepedino, 1981). Fruits (pepos) are smooth round gourds about 70 mm in diameter and contain approximately 250 seeds, on average (Kohn, 1989).

Linear population structure, relatively small numbers of plants (ramets) within patches, and the potential that many or all ramets within a patch represent clonal copies of the same genotype make *C. foetidissima* particularly amenable to studies of gene flow by pollen. Where alleles occur among the adult (flowering) plants in one patch that do not occur in neighboring patches, gene flow by pollen can be measured by genetical assay of seeds produced. If, as we find in this study, patches contain only one or a few genetic individuals, such situations may be common, allowing for the measurement of gene flow into a number of different patches. Because gene flow rates in other plants have been shown to vary greatly from patch to patch (reviewed by Ellstrand, Devlin, and Marshall, 1989), measurement of gene flow in a large number of patches is required to begin to estimate average rates of pollen-mediated gene flow.

MATERIALS AND METHODS

All flowering buffalo gourd plants growing along a 22-km section of U.S. 80 from Granite Pass, New Mexico to 6.5 km north of the Arizona state line, and along the section of Portal Rd. between Stateline Rd. and U.S. 80 (Fig. 1) were marked with numbered aluminum tags nailed into the soil near the root crown. In this area almost all buffalo gourd plants grow between the pavement edge and the barbed wire fence delimiting the right of way. This appears to be due both to the increased water availability caused by pavement runoff and to the exclusion of cattle which frequently graze *C. foetidissima* when other forage is scarce. Nearby habitat was searched, and the closest known patches away from the highway are indicated in Fig. 1. None is within 2 km of the highway, and in all cases, the closest patch to any roadside patch is another roadside patch.

In August of 1985 one or two fruits were collected from every ramet bearing fruit in the study area. Seeds from these fruits were assayed electrophoretically to identify cases where polleniferous plants in one or more neighboring patches possessed alleles that did not occur in hermaphroditic plants from a target patch. Such situations were then exploited for the study of interpatch gene flow. In August 1986, one to five fruits were collected from all individuals bearing fruit in patches where interpatch gene flow was to be assessed. Fruits were transported to the University of Pennsylvania where seeds were removed, dried for 2 days at 40 C, and stored in the dark at room temperature.

For electrophoresis, seeds were soaked overnight with seed coats removed and crushed in phosphate grinding buffer (Soltis et al., 1983). The grindate was absorbed onto filter paper wicks (Whatman #3) which were inserted into 12% starch gels. Two buffer systems were used: tris-EDTA-borate pH 8.6 (Soltis et al., 1983), 6 hr at 300 v and 30 mA, for alcohol dehydrogenase (*ADH-2*), and morpholine-citrate pH 6.1 (Shields, Orton, and Stuber, 1983), 6 hr at 250 v and 40 mA, for glutamate-oxaloacetate-transaminase (*GOT-1*), phospho-gluco-mutase (*PGM-2*), and phospho-gluco-isomerase (*PGI-2, PGI-3*). Stain recipes for *PGI* and *PGM* were from Soltis et al. (1983), for *ADH* from Vallejos (1983), and for *GOT* from Harris and Hopkinson (1976). Three alleles were observed at *GOT-1* and *ADH-2,* while all other loci were diallelic. For *GOT, PGI,* and *PGM,* numbering of loci and genetic interpretation of banding patterns are consistent with Kirkpatrick, Decker, and Wilson (1985) with the exception that both *PGI-2*

and *PGI-3* in *C. foetidissima* were diallelic. For *ADH*, zygomorphs were similar to those pictured in Orten (1983). For 1985 fruits, usually eight seeds were sampled from a single fruit from each individual in order to locate situations favorable to the detection of interpatch gene flow. For 1986 fruits, usually 20 seeds per fruit were sampled, and the data were used to assess the amount of pollen-mediated gene flow between patches.

Since maternal tissue (pericarp) did not stain for the enzymes that were polymorphic in seeds, maternal genotypes were inferred from seed genotypes. For hermaphrodites, assignment of maternal genotype was virtually unambiguous. Those scored as heterozygotes always bore some seeds that were homozygous for each alternative allele. Hermaphrodites scored as homozygous always produced a preponderance of seeds that were homozygous for a particular allele and never produced seeds homozygous for another allele. The high proportion of seeds homozygous for a particular allele made misassignment of genotype highly unlikely (P always < 0.01, $P < 0.001$ for all but three ramets).

For females however, genotypes could not always be assigned. Often about half the seeds were homozygous for a certain allele while half were heterozygous. This could occur either because the female was homozygous and received pollen from a heterozygous source, or vice versa. Thus in this study we concentrate on gene flow among hermaphrodites. We report on gene flow in females from three patches where genotypes of females at loci diagnostic for interpatch gene flow could be assigned unambiguously. Females inferred as heterozygous always produced some seeds homozygous for each allele. Females inferred as homozygous always produced at least one fruit from which two or fewer seeds in 20 were heterozygous.

Interpatch gene flow was assessed by the occurrence in seeds of nonmaternal alleles that could not have been produced by hermaphrodites within the patch. Sometimes only a fraction of the pollen produced from the nearest identifiable pollen source carried alleles detectable as interpatch gene flow, while the rest were "cryptic" in the sense that pollen produced within the target patch could carry the same alleles. To estimate the number of seeds sired by cryptic interpatch pollen we multiplied the number of detected interpatch matings by the ratio of cryptic to apparent gametes produced by the nearest identifiable pollen source. Detected plus cryptic matings were summed to estimate total interpatch gene flow by pollen. This method will underestimate total gene flow if cryptic pollen arrives from more distant sources. For three patches (A, B, and AA, Table 1) all pollen produced in the nearest patch was cryptic. Gene flow into these patches measures pollen flow from at least two (patches B, and AA) or three (patch A) patches away.

Heterogeneity among fruits from a patch in the frequency of seeds sired by interpatch pollen was assessed using log-linear analysis (JMP® version 2.01b, SAS Institute, 1989). Rank correlation analysis of the association of interpatch distance with the rate of interpatch gene flow was performed using Statview 512+ (Abacus Concepts Inc., 1986). Nonparametric analysis was used because the data could not be transformed to meet the assumptions of regression. Regression analysis on untransformed data gave similar results.

TABLE 1. *Distance to interpatch pollen source, number of ramets, number of genets, and the percentage of seeds of hermaphrodites and females sired by interpatch pollen in 13 patches of* Cucurbita foetidissima

Patch	Distance (km) to nearest identifiable pollen source	Ratio of cryptic to apparent gametes from nearest pollen source	Ramets (genotypes)	Seeds (fruits)	Number sired by extra-patch pollen	Percent sired by extra-patch pollen	Percent sired corrected for cryptic gametes
Hermaphrodites:							
A	3.1	1:3	6 (1)	134 (10)	0	0	0
B	1.7	1:3	1	24 (1)	0	0	0
C	0.48	1:3	2 (2)	75 (4)	10	13.3	17.7
D	0.22	1:1	7 (1)	176 (9)	12	6.8	13.6
O	0.14	0:1	1	59 (3)	3	5.1	5.1
P	0.14	0:1	4 (1)	146 (8)	5	3.4	3.4
Y	0.08	0:1	1	90 (5)	0	0	0
Z	0.08	0:1	1	20 (1)	3	15.0	15.0
AA	0.15	0:1	1	20 (1)	2	10.0	10.0
CC	0.28	1:1	6 (1)	120 (6)	2	1.7	3.4
DD	0.03	1:3	1	60 (3)	29	48.3	64.4
EE	0.28	1:1	2 (1)	40 (2)	0	0	0
FF	0.32	1:7	8 (2)	160 (8)	11	6.9	7.9
Mean						8.5	10.8
Females:							
O	0.14	0:1	2 (2)	47 (2)	6	12.7	12.7
Y	0.08	0:1	6 (2)	150 (10)	13	8.6	8.6
EE	0.28	1:1	5 (1)	100 (5)	40	40.0	40.0[a]
Mean						20.4	20.4

[a] Extrapatch sire was most likely patch LL, which does not produce cryptic gametes (see Results).

RESULTS

Overall, 6.9% (77 of 1,124) of the seeds of hermaphrodites must have resulted from interpatch pollen flow (Table 1). The longest distance between pollen donor and recipient detected was 0.7 km (pollen from patch E siring seed from patch C). From 0 to 48% ($\bar{X} = 8.5\%$) of the seeds assayed from a patch must have been sired by pollen from outside the patch. If cryptic gametes from the nearest identifiable pollen source are accounted for, the mean rate of gene flow into patches increases to 10.8%. The highest rate of gene flow was detected in seeds from patch DD, comprised of a single small individual only 30 m from the large patch CC. However, there was no overall relationship between rates of gene flow among hermaphrodites and distance (Kendall's $t = -0.26$; $P \gg 0.1$, two-tailed). Of the 77 seeds that must have been sired by interpatch pollen, 69 (90%) could have been sired by the nearest identifiable pollen source while the remaining must have resulted from gene flow from more distant sources.

We examined among-fruit patterns of interpatch matings within the five patches where five or more cases of interpatch mating were detected. Significant heterogeneity among fruits in four of those patches was detected (all significant likelihood tests $P < 0.01$). For example, of the ten seeds from patch C sired by interpatch pollen, nine came from a single fruit while only one was found in seeds from the three other fruits examined.

All hermaphrodites shared the same five-locus genotype in five of the seven patches that contained more than one hermaphroditic ramet. In each of these five patches the

shared genotype was heterozygous at one or more of the electrophoretic loci. This suggests that clonal propagation may be the predominant mode of reproduction within patches. In the other two patches containing multiple hermaphrodites (patches C and FF, Table 2), two genotypes occurred. In both cases, one of the genotypes could have resulted from offspring formed by self-fertilization of the other genotype of hermaphrodites in the patch. That is, one of the genotypes present contained a subset of the alleles found in the other genotype in that patch.

Seeds from females were analyzed from three patches, each of which contained at least one hermaphroditic plant (Table 1). Overall, 20% (59 of 297) of the seeds from these females must have been sired by pollen from outside the patch. Significant among-fruit heterogeneity in interpatch siring of seeds of females was detected in all three of the patches examined ($P \leq 0.01$ in all cases). In the most striking case, three of the fruits examined from patch DD could have been sired almost entirely (59 of 60 seeds) by local pollen, while the other two fruits likely received little or no local pollen. In these two fruits, only one of 40 seeds examined could have been locally sired, and the same pollen genotype can be produced in nearby patches. It is possible that the flowers giving rise to the latter two fruits bloomed earlier than the hermaphrodites in the patch. Based on seed genotypes, pollen from patch LL is the likely sire of these two fruits. The small number of patches examined and high heterogeneity among fruits within patches precludes formal statistical comparison between rates of successful interpatch pollen received by females and hermaphrodites. However, in each of the three patches, rates of interpatch siring among seeds of females exceeded rates of interpatch siring found among seeds of hermaphrodites.

DISCUSSION

Rates of pollen-mediated gene flow into 13 patches showed high variation among patches (0 to near 50%), with an estimated average rate of about 11% for seeds of hermaphrodites and 20% for seeds of females. While the highest rate of gene flow was associated with the smallest interpatch distance, variation in rates of interpatch gene flow was not significantly associated with interpatch distance. The linear population structure might suggest gene flow would follow a stepping stone model (Kimura and Weiss, 1964) with exchange restricted to matings between neighboring patches. However, in 10% of the instances of apparent gene flow documented in this study, pollen must have come from a more distant source.

Ellstrand, Devlin, and Marshall (1989), summarizing work on *Raphanus sativus,* reported that rates of gene flow into seven patches varied from 3.2% to 18% ($\bar{X}$ = 10%). In their study, interpatch distances ranged from 0.1 to 1.0 km. As in our study, Ellstrand, Devlin, and Marshall (1989) also found no relationship of isolation distance on gene flow into patches. Rates of pollen-mediated gene flow between small patches of cultivated *Cucurbita pepo* and wild *C. texana* ranged from 0% to 15% ($\bar{X}$ = 5%, Kirkpatrick and Wilson, 1988). While the rates of interpatch gene flow reported here are similar, we agree with previous statements (Ellstrand and Marshall, 1985; Ellstrand, Devlin, and Marshall, 1989) that rates of long-distance pollen flow may be so highly idiosyncratic from species to species and place to place that it is far too early for any generalizations. More work of this type is warranted.

All direct studies of pollen-mediated gene flow have concentrated, of necessity, on gene flow into relatively small patches. The studies of gene flow into patches of *Raphanus sativus* have concentrated on patch sizes generally <60 plants. In the present study, numbers of ramets and genets per patch were far fewer. However, from the pollinator's point of view, patches of the two species may represent similar amounts of resources spread over comparable areas since both plants and flowers of *C. foetidissima* are extremely large in comparison to *Raphanus.* Thus while the consequences of gene flow would be quite different for each species, the fact that average rates of gene flow are similar may indicate some consistency in this parameter among relatively small patches of insect-pollinated plants.

While methods of paternity exclusion are currently inapplicable to the measurement of gene flow into large populations, some lines of evidence indicate that rates of gene flow in such situations may also be substantial. Inferential methods of measuring gene exchange between populations (Slatkin, 1985a, b, 1987; Slatkin and Barton, 1989) have indicated rates of exchange between 0.5 and 5.5 individuals per generation (Slatkin, 1985b; Soltis and Bloom, 1986; Golenberg, 1987; Hamrick, 1987). Such exchange rates, inferred for comparatively large populations, coupled with direct measures of high rates of gene flow into small populations, indicate that gene flow between patches may often be great enough to counteract the effects of genetic drift or weak selection (see also Devlin and Ellstrand, 1990).

Such high rates of long-distance gene flow would not be expected based on the leptokurtic distributions of pollinator flight distances and pollen-mimicking dye movements recorded in several studies (see Levin, 1984 for review). We documented gene flow due to squash bee pollinators at distances up to at least 0.7 km. Gene flow between *C. pepo* and *C. texana* pollinated by *Xenoglossa angustior* and *Peponapis pruinosa* was recorded at distances up to 1,300 m (Kirkpatrick and Wilson, 1988). Such long-distance gene flow is apparently not restricted to plants pollinated by specialist bees. In *Raphanus* visited by generalist pollinators such as *Apis mellifera,* small lepidoptera, and syrphid flies, gene flow at distances up to 1,000 m has been detected (Ellstrand and Marshall, 1985; Ellstrand, Devlin, and Marshall, 1989).

We found significant variation in gene flow among fruits arising from the same patch. A similar pattern of interfruit variation was noted by Kirkpatrick and Wilson (1988). Thus the arrival of long-distance pollen appears not only unpredictable with respect to isolation by distance, but, within patches, it is clumped with respect to stigmas. Such patterns could result from differences in the available pollen pool across blooming days or differences within days in the distribution of intra- and interpatch pollen on stigmas. Ellstrand and Marshall (1985) recorded heterogeneity among plants in the proportion of seeds sired by interpatch pollen but could not reject the possibility that this heterogeneity was an artifact of variation among plants in the discriminatory power of paternity exclusion anal-

ysis (see also Devlin and Ellstrand, 1990). The heterogeneity found in our study could not have resulted from differences in the power to detect interpatch matings since the variation usually occurred between flowers of the same plant or genotype.

Although we documented substantial gene flow between patches via pollen movement, about 90% of the seeds of hermaphrodites appear to be sired by pollen from within the patch. Hermaphroditic plants within a patch often share the same genotype, presumably due to vegetative reproduction. There are seldom more than two genets in a patch (Table 1). Most seeds of hermaphrodites, therefore, appear to result from self-fertilization. In the related taxa *C. pepo* and *C. texana,* Kirkpatrick and Wilson (1988) showed that even when plants of different genotypes were grown next to one another along a row, seeds were largely self-fertilized.

While this study suggests a high selfing rate for seeds of hermaphrodites, estimation of the actual selfing rate (including within-patch matings between different genets) awaits application of a mating system model (Clegg, 1980; Ritland and Jain, 1981; Ritland, 1985, 1986) appropriate for such a highly structured and clonal population. However, the interpretation that most seeds of hermaphrodites result from geitonogamous (self-) fertilization is supported by data on seed set and seedling performance following self-, cross-, and natural pollinations of hermaphrodites. Seed sets following self- and natural pollination did not differ, while hand cross-pollinations produced significantly more seeds (Kohn, 1988), suggesting that a large fraction of the seeds of hermaphrodites result from self-fertilization (see Charlesworth, 1988). Under field conditions, seedlings derived from either cross-pollination of hermaphrodites or natural pollination of females survived three times more frequently than seedlings derived from either self- or naturally pollinated seeds from hermaphrodites (Kohn, 1988). Thus, if self-fertilization is the cause of poor survival of seeds from hermaphrodites, their seeds must be mostly self-fertilized.

While most seeds from patches containing hermaphrodites appear to be sired by pollen from within the patch, female plants in all-female patches, separated from the nearest pollen source by up to 0.5 km, routinely set fruit (J. R. Kohn, personal observation). Since buffalo gourd does not produce seeds by apomixis (Ordway et al., 1987), pollen flow at this distance must be fairly common. For patches containing hermaphrodites, pollen produced within the patch must swamp interpatch pollen.

The fraction of interpatch pollen on stigmas may be a decreasing function of pollen production within the patch. All-female patches must produce seeds sired solely by interpatch pollen. Small solitary hermaphrodites may receive a higher fraction of interpatch pollen on their stigmas than hermaphrodites in large patches where pollen production is high. Thus while clonal propagation may enhance the longevity and size of a genotype, it may also lower the frequency of outcrossing, and, at least in this species, the fitness of seeds produced by that genet (Handel, 1985).

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