Is gene flow the most important evolutionary force in plants?[1]

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Although theory has demonstrated rather low levels of gene flow are sufficient to counteract opposing mutation, drift, and selection, widespread recognition of the evolutionary importance of gene flow has come slowly. The perceived role of gene flow as an evolutionary force has vacillated over the last century. In the last few decades, new methods and analyses have demonstrated that plant gene flow rates vary tremendously—from nil to very high—depending on the species and specific populations involved, and sometimes over time for individual populations. In many cases, the measured gene flow rates are evolutionarily significant at distances of hundreds and sometimes thousands of meters, occurring at levels sufficient to counteract drift, spread advantageous alleles, or thwart moderate levels of opposing local selection. Gene flow in plants is likely to often act as a cohesive force, uniting individual plant species into real evolutionary units. Also, gene flow can evolve under natural selection, decreasing or increasing. The fact of frequent, but variable, plant gene flow has important consequences for applied issues in which the presence or absence of gene flow might influence the outcome of a policy, regulatory, or management decision. Examples include the unintended spread of engineered genes, the evolution of invasiveness, and conservation. New data-rich genomic techniques allow closer scrutiny of the role of gene flow in plant evolution. Most plant evolutionists now recognize the importance of gene flow, and it is receiving increased recognition from other areas of plant biology as well.

Key words: biological species concept; conservation; dispersal; gene flow; hybridization; immigration; local adaptation; migration; population genetics; transgenes.

Some scientists consider the word "evolution" to be more or less equivalent with "natural selection" or adaptation. They would, of course, be wrong. Biological evolution is the change of allele frequencies in a population over time (Wilson and Bossert, 1971).

Evolutionary change proceeds via four different phenomena, often called "evolutionary forces" or "evolutionary mechanisms": mutation, selection, drift, and gene flow. Working separately or together, they affect the change of allele frequencies in a population from generation to generation. The relative role of each of these evolutionary forces has been the topic of lively discussion in evolutionary biology for over a century.

In particular, the importance of gene flow, the successful movement of genes among populations, has been controversial. The perception of the evolutionary role of gene flow has vacillated from critical to trivial back to conditionally critical. For example, while the annual number of articles in *American Journal of Botany* using the term "gene flow" has generally increased in

the last 50 yr, the increase has been far from monotonic (Fig. 1). Furthermore, different evolutionists, even contemporaries, have had radically different views of the importance of gene flow. Discussion of gene flow in evolution textbooks of the mid-20th century ranged from thorough (e.g., Grant, 1963) to almost nil (e.g., Stebbins, 1966). Interestingly, the same is true for the first decade of the 21st century, (e.g., Futuyma, 2009 vs. Zimmer, 2010).

The mixed respect gene flow has received over the years is puzzling. The mathematical theory of population genetics first developed by luminaries such as Haldane and Wright (reviewed by Slatkin, 1985a, 1987) reveals gene flow as a potentially significant evolutionary force. Those theoretical expectations are summarized in Table 1. Surprisingly low levels of gene flow are sufficient to counteract opposing mutation, drift, and selection (for detailed discussion, see Slatkin, 1985a, 1987; Ellstrand, 2003a).

At sufficient levels, gene flow tends to homogenize populations, especially in opposing drift. Note that "homogenization" in the sense that the evolutionary theorists have intended it is not panmixia but instead the opportunity for most alleles to be present in most populations (contrast Wright, 1969 vs. Waples and Gaggiotti, 2006). Starting in the 1940s, the evolutionists of the Modern Synthesis (e.g., Mayr, 1942) generally considered gene flow to be the evolutionary glue that held species together. Mayr (1963, p. 521) estimated that "normal gene flow is at least as high as 10^{-3} to 10^{-4} (per generation) for open populations". The high end of this minimum estimate sets gene flow at a rate much more than an order of magnitude larger than that of mutation. Consequently, he (Mayr, 1963) reasoned gene flow should be the primary source of genetic variation for any given population. But these conclusions were based on largely anecdotal empirical evidence about gene flow rates.
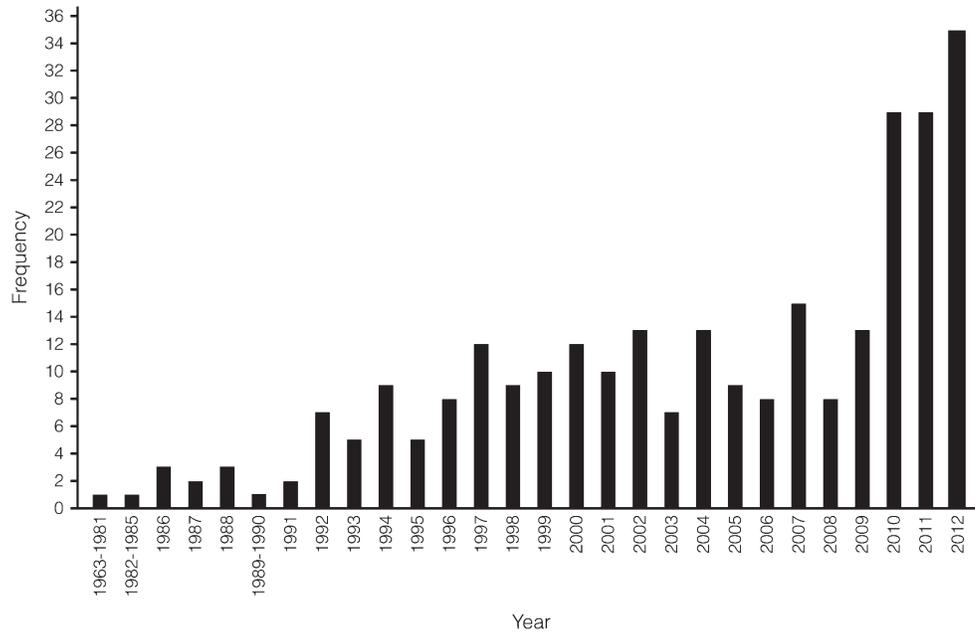
Fig. 1.   Frequency of papers using the term "gene flow" in the *American Journal of Botany* from 1962 through 2012 (based on a Web of Science search).

Research relevant to gene flow moved beyond the anecdotal. Quantitative studies of animal and plant dispersal slowly accumulated, usually measurements of the movements of individuals, pollen, or seeds. The data collected were sufficient to call the ubiquity of gene flow into question. In 1969 Ehrlich and Raven published a short watershed paper reviewing some of the new data and discussing its significance. They concluded that "gene flow in nature is much more restricted than commonly thought"; for example, "in plants … there is considerable evidence that distances from 50 feet (15 m) to a few miles (several kilometers) may effectively isolate populations" (Ehrlich and Raven, 1969, p. 1229). They predicted "gene flow eventually might be discovered to play a rather insignificant role in evolution" (Ehrlich and Raven, 1969, p. 1231) and concluded that the apparent uniformity of species over space has more to do with stabilizing natural selection than cohesion under gene flow.

But Ehrlich and Raven (1969) offered scant information about actual gene flow rates. Their five-page paper relied mostly on studies of animal, seed, and pollen dispersal. They cited only two papers that measured allele movement, rather than gross dispersal.

Determining the evolutionary significance of gene flow awaited a more thorough review, with more data on actual gene movement to estimate rates. The high end of the gene flow levels predicted by Mayr would be sufficient to overwhelm drift, most typical mutation rates, and modest levels of opposing selection while being sufficient to speed the spread of advantageous alleles. In stark contrast, to fit Ehrlich and Raven's prediction that gene flow is insignificant, gene flow rates would have to be considerably lower.

Ehrlich and Raven's data came largely from plants. Plants are well suited for gene flow studies. Empirical gene flow research on seed plants—both descriptive and experimental studies—is easy because individuals are typically stationary while their seed and pollen are mobile. Furthermore, numerous simple Mendelian markers became available for dozens of species of plants long before the advent of biochemically based genetic markers. Plants (in particular, seed plants) became the organisms of choice in the mid-20th century to estimate gene flow rates, and they continue to play a prominent role today (Ashley, 2010).

Indeed, until biochemical genetic markers were routinely used for measuring population genetic structure, plants were almost the sole source of actual gene flow rates. This review first addresses the history of plant gene flow research. The search for generalities eventually resulted in the current view of gene flow: plant gene flow varies but is frequently high enough to be evolutionarily significant. The review continues by considering the consequences of the current view for both plant evolution as well as applied plant evolutionary biology. The review concludes with a look to the future with regards to newly emerging research tools as well as questions ripe for study.

TABLE 1.   Recurrent gene flow vs. the other evolutionary forces (adapted from Ellstrand [2003a]).

Gene flow vs. …	When gene flow is of consequence
Drift (neutral gene flow)	One to a few gene flow events per generation tends to homogenize populations, independent of population size. In that case, gene flow acts as a cohesive force.
Negative selection (detrimental gene flow)	Gene flow counteracts opposing selection of equal or smaller magnitude. In that case, gene flow acts as a force that prevents evolution of local adaptation.
Positive selection (beneficial gene flow)	Gene flow augments selection to speed the geographic spread of a favored allele. In that case, gene flow in concert with selection acts as a creative force.
Mutation	Gene flow at a rate greater than the mutation rate tends to overwhelm mutation effects. In that case, gene flow, rather than mutation becomes the immediate primary source of genetic diversity for a population.

Gene flow in plants is clearly an important component of basic and applied plant biology that cannot be ignored.

THE RISE OF PLANT GENE FLOW RESEARCH

Gene flow in plants can be accomplished by cross-fertilization or by the dispersal of diaspores such as whole plants, plant fragments, seeds, and spores (Ellstrand, 2003a). For the purposes of this review, the emphasis is on those plants where most of the research has been conducted, seed plants.

Gene flow among conspecific populations played little role during the early growth of plant evolutionary biology. For example, G. L. Stebbins, often acknowledged to be botany's Modern Synthesis leader, rarely considered gene flow. His research never focused on gene flow per se. He used the phrase "gene flow" in only a single journal article (Stebbins et al., 1947) and "migration" in five others. Furthermore, he made no mention of gene flow in his evolution textbook (Stebbins 1966). Indeed, I met with Stebbins when he visited Riverside in the early 1980s, and he characterized gene flow research as "uninteresting".

But even if Stebbins was unenthusiastic about intraspecific gene flow, he had considerable interest in interspecific gene flow, that is, hybridization and introgression (Rieseberg and Yakimowski, in press). Hybridization played a role in dozens of his papers. He also acknowledged that introgression—the incorporation of alleles from one taxon mixed into another in the generations after hybridization—could provide an important substrate for future adaptive evolution (Stebbins, 1959, 1969).

Introgression's champion, Edgar Anderson, probably thought more about gene flow than any other botanist in the infant days of plant evolutionary biology. Many of the species-to-species gene migration concepts developed in Anderson's seminal monograph, *Introgressive Hybridization* (Anderson, 1949), apply equally well to population-to-population gene movement. In particular, Anderson believed that the introgression of alleles from one species to another had great potential as a creative stimulus for evolution (e.g., Anderson and Stebbins, 1954). Thus, the perception of gene flow's role in the botanical Modern Synthesis was largely subsumed within the enthusiasm for hybridization and introgression (see Rieseberg and Yakimowski [in press] for more detail.)

Up to the time of Ehrlich and Raven's (1969) paper, plant gene flow research was primarily the province of plant breeders, from agronomists to foresters. Breeders' concern was "pollen contamination", the frustration of selective gain under field conditions due to extraneous pollination. If, for example, a field-corn breeder's plot was too close to that of a popcorn or sweet-corn breeder, the unintentional production of intercultivar hybrid seed created havoc for both selecting for new varieties and multiplying seed for the market. Increasing isolation distance was the most popular approach to reproductively isolate experimental plants. Thus, breeders needed to determine distances sufficient to reduce contamination of compatible pollen to reasonable levels (Kernick, 1961).

To estimate the relationship of intervarietal cross-fertilization with distance, breeders often planted experimental stands with a core source population of plants homozygous for a dominant marker surrounded by individual recipient (sink) plants homozygous for the recessive allele at a range of distances from the core. Figure 2 displays the layout of an experiment of this type. Testing the progeny of the sink plants for the dominant phenotype revealed the relationship between cross-pollination rate (gene flow) and distance. With such data, agricultural scientists could assign crop-specific rule-of-thumb "isolation distances" to reduce pollen contamination to tolerable levels. Studies of this type by Bateman (1947a, b) using *Beta vulgaris* L. (beet), *Brassica rapa*
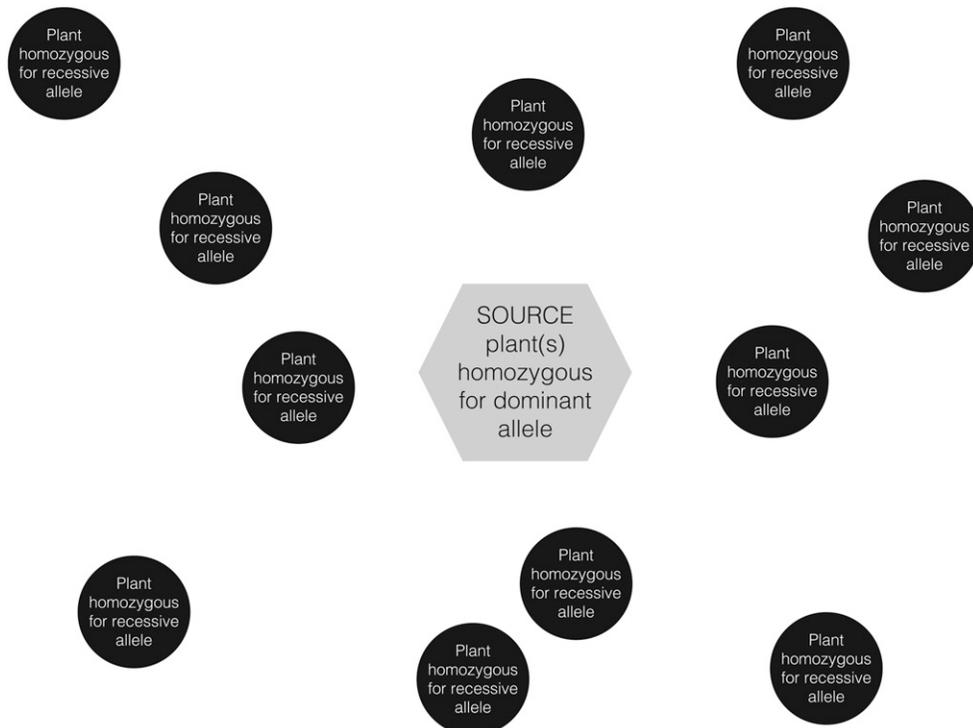

Fig. 2.   Schematic of experimental design for measuring allele dispersal from a source.

L. (turnip), *Raphanus sativus* L. (radish), and *Zea mays* L. (corn), revealed that cross-fertilization drops off rapidly with distance. In fact, Bateman's studies were used as the few examples of actual gene flow by Ehrlich and Raven (1969).

If Ehrlich and Raven's short paper sought to put gene flow into its coffin, Levin and Kerster's (1974) nearly 100-page *tour de force* nearly nailed the coffin closed. After reviewing the extant theory and data, they concluded with the same prevailing view of other contemporary plant evolutionary biologists who supporting Ehrlich and Raven's view of highly restricted gene flow (e.g., Grant, 1971; Bradshaw, 1972). Dealing only with seed plants, Levin and Kerster (1974) reviewed hundreds of studies dealing with estimates of how pollen and seed dispersal vary with distance—which they deemed "potential gene flow" – as well as the "incidence of fertilization (in the case of pollen) and establishment of reproductive individuals (in the case of seeds) as a function of distance" (Levin and Kerster, 1974, p. 140), which they named "actual gene flow". They evaluated as many dispersal distributions as possible. Many of those distributions were based on pollen contamination studies described above, but other kinds of dispersal studies were beginning to be conducted by both the authors themselves and by other plant population biologists, such as measuring plant-to-plant pollinator flight distances as surrogates for interplant crossing distances (e.g., Levin and Kerster, 1968).

The vast majority of the dispersal curves were highly skewed toward the source; that is, the great majority of pollen or seed involved in both potential and actual gene flow ended up a short distance from the source plant or plants, typically a few meters or less. Likewise, the dispersal curve's shape was characteristically leptokurtic; that is, it deviated from normality with a higher peak and fatter, longer tail. Some typical dispersal curves are featured in Fig. 3.

Levin and Kerster (1974) also conducted an extensive examination of the relevant population genetic theory. Given the apparent ubiquity of highly limited dispersal, they paid particular attention to theory about the consequences of restricted gene flow, such as Wright's (1943, 1946) models of isolation by distance and his concept of neighborhood, that is, the spatial extent around an individual in a population within which mating is random. Combining their review of the current plant data with the theory, Levin and Kerster (1974, pp. 202–203) concluded, "The level of gene flow in most species must average less than 0.01 between populations or population subdivisions a few hundred meters apart, and at least two orders of magnitude less between populations a mile or more apart… Whatever the actual movement of pollen and seeds in natural and artificial populations, it is sufficiently restricted as to be overridden by natural selection" and sometimes by drift.

However, by that time, descriptive population genetic studies with allozymes as genetic markers revealed unexpectedly high levels of intrapopulation variation in a variety of species, including plants. How, then, to explain why drift and various kinds of purifying selection had not purged these widespread polymorphisms from plant populations? Levin and Kerster (1974, pp. 208–209) asked and answered the same question. "What are the sources of alleles for populations well isolated by distance? They are mutation, and more importantly, introgressive hybridization. … In many genera we surmise that interspecific gene exchange occurs at a greater level than interpopulation hybridization when conspecific populations are widely spaced and related species are biotically sympatric."

*Inter*species gene flow at higher rates than *intra*species gene flow? That statement was a surprising suggestion. At that time,

introgression was no longer getting a lot of respect. The examples used with heady enthusiasm by Anderson and his disciples were receiving renewed scrutiny with the newly available biochemical genetic markers. Such research revealed clinal changes in morphological traits originally attributed to introgression often could be better explained by environmental factors (e.g., von Rudloff et al., 1967). Furthermore, estimates of natural interspecific hybridization were nearly nonexistent in the middle of the 20th century; thus, they were presumed to be extremely low. Nonetheless, given the apparent absence of within-species gene flow at any meaningful distances, how else could anyone explain high levels of intrapopulation variation?

The perception that plant gene flow was evolutionarily insignificant persisted for about a decade. Levin (1981, p. 233) summarized the sentiment of the era: "Even if gene flow distances were twice as large as we now think, the spatial scale of gene dispersal: (1) is still small enough to allow spatial differentiation over short distances with moderate selective differentials, and (2) is too small to be a major cohesive force within a species". A few years after making that statement, and a decade after his important review with Kerster, Levin (1984, p. 243) characterized the likely plant immigration rate "to be much less than 1%". (Note that while this view was the dominant one at this time; it was not universal [e.g., Antonovics, 1968a].)

But soon after, two types of data emerged that challenged and eventually rejected the hypothesis that gene flow in plants was universally evolutionarily insignificant. In both cases, the data first came from scientists who had originally been addressing other issues in evolutionary biology.

Parentage studies—In the 1980s, several plant geneticists independently started conducting various "paternity"-type analyses with the goal of measuring the likes of male fitness, gender, multiple paternity, and interplant mating patterns (e.g., Ellstrand, 1984; Meagher, 1986; Schoen and Stewart, 1987). Working with presumably "isolated" populations, some researchers ended up discovering a fraction of seeds could not have been sired by any local father, identifying seeds that must have been sired by plants in another population—the result of gene flow by pollen. The recognition that interplant mating—both within and among populations—could be measured directly via the genetic analysis of progeny encouraged other studies in experimental and natural populations. An increasing number of those reported mating rates between spatially isolated populations (Ashley, 2010).

Marker-based gene flow studies continue today. Paternity methodologies have evolved. DNA-based markers have replaced enzyme-based markers. Microsatellites are the marker *du jour*. Compared with other codominant Mendelian markers, microsatellites are relatively accessible and inexpensive for most species. With multiple polymorphic loci, each averaging many alleles, they afford considerable exclusion information (Selkoe and Toonen, 2006; Ashley, 2010). Methods for assigning paternity have also evolved. Simple exclusion by inspection has been replaced by an array of maximum likelihood and Bayesian methods that can take advantage of the rich DNA-based data sets (e.g., Smouse and Sork, 2004; Hadfield et al., 2006). Likewise, it was recognized that certain long-distance mating events might be erroneously attributed to local pollen donors. Thus, methods to correct for such "cryptic gene flow" were developed (e.g., Devlin and Ellstrand, 1990).

Table 2 provides a selected, but largely representative, list of estimates of gene flow via interpopulation mating from paternity
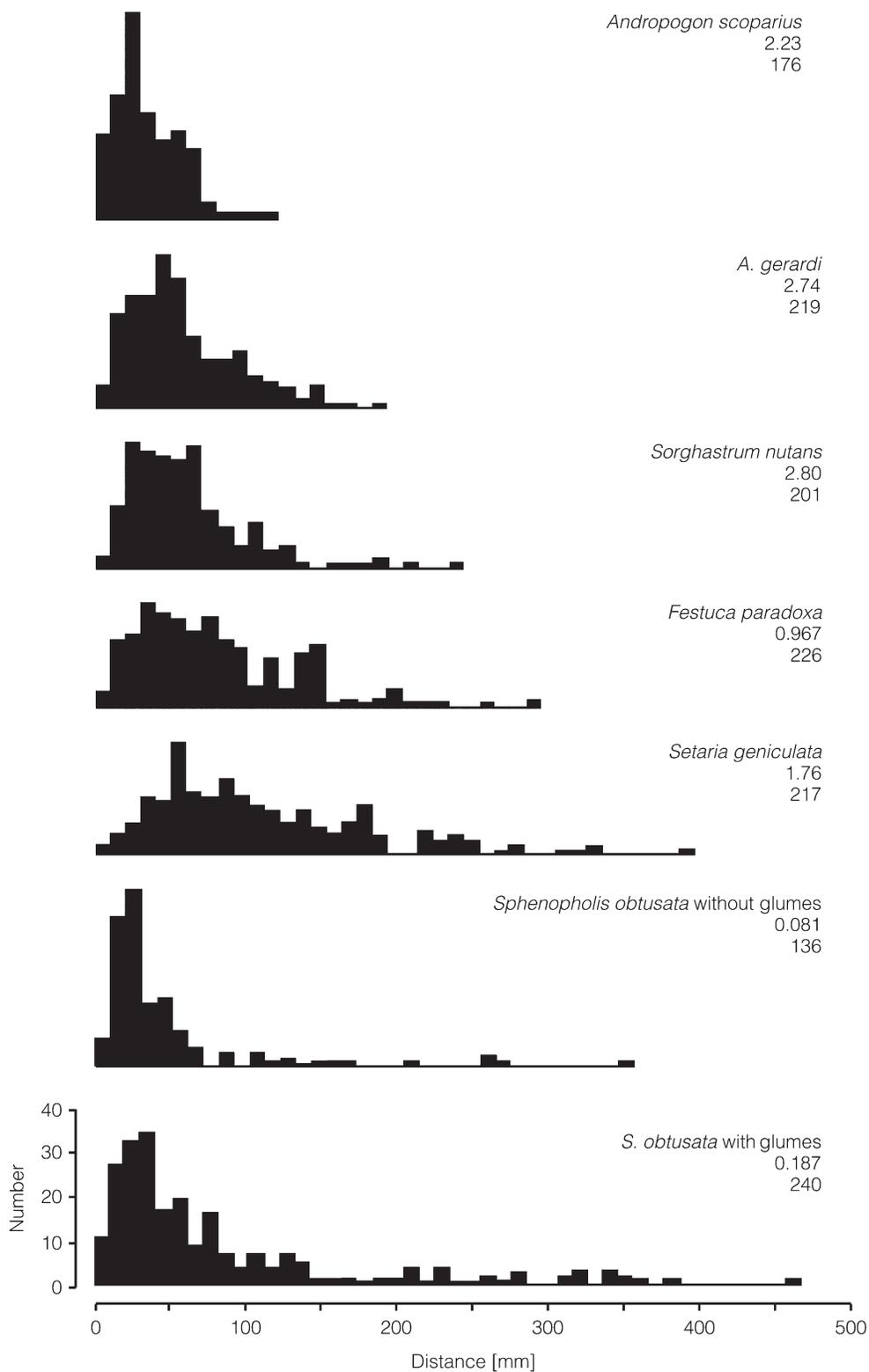
Fig. 3. Significantly right-skewed and leptokurtic propagule dispersal distributions for seven Missouri prairie grass species. Propagules were dropped from a height of 1 m in still air. Distance refers to the unidirectional, axial distance from the source (adapted from fig. 2 of Rabinowitz and Rapp [1981]).

TABLE 2. Selected estimated interpopulation mating rates [a] from paternity studies in natural plant populations (adapted and updated from Ellstrand [2003b]).

Species and Population designation	Minimum distance to possible male parent (m)	Interpopulation mating rate [a] estimate (%)	Reference
Araucaria angustifolia	1700	10	Bittencourt and Sebbenn, 2007
Calothamnus quadrifidus			Byrne et al., 2007
B	633	37.5	
K	2180	9.7	
L	2180	42	
M	260	5.0	
N	2210	42.8	
T	2800	0	
Cucurbita foetidissima			Kohn and Casper, 1992
Patch A (1 ♀+♂ genet)	3100	0	
Patch B (1 ♀+♂ genet)	1700	0	
Patch C (2 ♀+♂ genets)	480	17.7	
Patch D (1 ♀+♂ genet)	220	13.6	
Patch O (2 ♀ genets; 1 ♀+♂ genet)	140	12.7♀; 5.1♀+♂	
Patch P (1 ♀+♂ genet)	140	3.4	
Patch Y (2 ♀ genets; 1 ♀+♂ genet)	80	8.6♀; 0.0 ♀+♂	
Patch Z (1 ♀+♂ genet)	80	15.0	
Patch AA (1 ♀+♂ genet)	150	10.0	
Patch CC (1 ♀+♂ genet)	280	3.4	
Patch DD (1 ♀+♂ genet)	30	64.4	
Patch EE (1 ♀ genet; 1 ♀+♂ genet)	280	40 ♀; 0.0 ♀+♂	
Patch FF (2 ♀+♂ genets)	320	6.9	
Datisca glomerata	500	27.0	Nazakato et al., unpublished data, cited in Rieseberg and Burke, 2001
Eucalyptus wandoo			Byrne et al., 2008
F	350	48.7	
J	1000	44	
K	180	64.2	
R	350	64.8	
S	580	33.3	
Ficus sycamorus			Ahmed et al., 2009
1 (isolated tree)	85 300	100	
3 (isolated tree)	24 200	100	
4 (isolated tree)	24 600	100	
6 (isolated tree)	71 400	100	
8 (isolated tree)	51 000	100	
17 (tree in a population)	14 200	100	
28 (tree in a population)	20 600	100	
40 (tree in a population)	27 900	100	
41 (tree in a population)	22 300	100	
Magnolia stellata	90	2.5	Setsuko et al., 2007
Pinus densiflora			Iwaizumi et al., 2009
2004	100	47.8	
2005 (same population, different years)	100	60.5	
Pinus flexilis	2000	6.5	Schuster and Mitton, 2000
Pinus sylvestris	30 000	4.3	Robledo-Arnuncio and Gil, 2005
Prunus malaheb	1500	9.5	García et al., 2005
Quercus macrocarpa			Craft and Ashley, 2010
Burnham Prairie	200	46	
Goose Lake	500	46	
Quercus robur	80 000	35	Buschbom et al., 2011
Raphanus sativus			Ellstrand et al., 1989
1982	1000	8.6	
1983	600	8.2	
1984	100	17.9	
1985A	200	9.8	
1985B	200	18.0	
1986A	150	4.0	
1986B (different populations)	150	3.2	
Swietenia humilis			White et al., 2002
Butus/Jicarito	1100	47.0	
Jiote	1100	38.3	
Tablas plains	1200	68.4	
Tree 501 (isolated tree)	1400	100	

[a] Because pollen is haploid and the maternal plant is local, the gene flow rate is half the value of the interpopulation mating rate.

studies conducted in natural populations. Note that because pollen is haploid, and the maternal plant is local, interpopulation mating rates reported in Table 2 represent the gene flow rate multiplied by two. The list includes a diverse set of temperate and tropical plant species varying in habit, breeding system, and pollination syndrome. The entries in Table 2 make it clear that gene flow in plants is not always frustrated by distances of 100 or even 1000 m. Nor are gene flow rates always in accord with Levin's (1984) prediction of "much less than 1%".

With enough genetic information, both paternity and maternity can be assigned to young plants or dispersed seeds. Such parentage assignment is especially feasible in populations of long-lived perennials where both parents and progeny are available for genotyping. Joint maternity and paternity assignment was originally applied to finding parents within a population (Meagher and Thompson, 1986, 1987). Studies reporting immigrant seeds and seedlings soon followed, as did more refined methods for assigning the most likely parents. Currently popular is the software CERVUS, which uses likelihood-based methods for both paternity and parentage assignment (Kalinowski et al., 2007). Not unexpectedly, parentage studies have typically involved long-lived woody species, usually trees. Only a small fraction of those studies are designed to measure seed immigration from other populations. Examples of seed immigration rates gleaned from parentage analysis are given in Table 3. Although seed gene flow rates by parentage analysis remain rare compared with pollen gene flow rates by paternity analysis, the species in Table 3 are heterogeneous with regards to pollination syndrome, breeding system, and seed dispersal syndrome. Table 3 reveals that gene flow by seed, much like gene flow by pollen, often occurs at a hundred meters or hundreds of meters and often at respectable rates.

Studies that measure gene flow by seed or pollen by identifying long-distance parents have certain limitations. By necessity, they focus either on small, relatively isolated, populations or on a set of spatially adjacent individuals that are part of a large population. Only the first approach is capable of measuring immigration from another population. As Ashley (2010, p. 151) put it, "One needs to sample and genotype … all of the candidate parents in an area relative to pollen or seed dispersal distance, which will simply not be possible for plants that occur in densities of tens to hundreds per square meter or thousands per hectare. The costs and time involved in genotyping are prohibitive and even microsatellites do not provide enough resolution to distinguish among thousands of candidate parents."

Small populations may have certain properties that influence gene flow receipt. For example, experimental studies have revealed that as population size gets smaller immigrant gene flow by pollen tends to increase (e.g., Klinger et al., 1992; Goodell et al., 1997). Thus, surveying smaller populations may measure gene flow rates that are higher than the mean for all of the populations of a species.

Another limitation to these parentage studies is that they measure interpopulation mating or seed immigration into a population. While they measure gene flow, they do not measure its generation-to-generation evolutionary impact on the population in the context of all of the evolutionary forces. For example, they do not measure the relative fate of immigrant seeds. Some reciprocal transplant experiments have revealed that seed from local parents generally have a fitness advantage over seed from other populations or sired by immigrant pollen (e.g., Levin, 1984; Montalvo and Ellstrand, 2000, 2001). While parentage studies that measure gene immigration are more informative than simple dispersal studies (e.g., recording the distances that pollinators move from plant-to-plant or measuring the seed shadow around an experimental plant), ideally, full-generation (seed-to-seed) or multigeneration approaches are necessary to describe the dynamics that result in evolutionary change (e.g., Grant and Grant, 2002). The next method involves a gene flow estimate that is derived from multiple generations of evolutionary change.

Spatial population genetic structure studies—The second challenge to the perception of highly limited gene flow in plants came from descriptive studies of population genetic structure as measured first by allozyme polymorphisms and later by DNA-based markers. The original goal of such studies was to measure levels of genetic polymorphism and heterozygosity within populations and within species (Lewontin, 1974). Soon, additional attention was given to how allelic differentiation was spatially structured among different populations. Since gene flow can play an important role in shaping a species' spatial genetic structure, it follows that the rate of gene flow can be inferred from the amount of genetic differentiation measured from the existing population genetic structure of marker loci, especially if those markers are selectively neutral (Selkoe and Toonen, 2006). Current population genetic structure represents the effects of the evolutionary forces that have worked on it over generations. Gene flow inferred from population genetic structure thus represents a historic rather than current value.

TABLE 3. Selected parentage studies in natural plant populations that identify the fraction of immigrant seeds or seedlings.

Species and Population designation	Minimum distance to next population (m)	Immigration (successful gene flow by seed) rate estimate (%)	Reference
Araucaria angustifolia	1700	0	Bittencourt and Sebbenn, 2007
Copaifera langsdorffii	12000	0	Sebbenn et al., 2011
Pinus densiflora			Iwaizumi et al., 2013
2003	100	25.2	
2004	100	26.2	
2005	100	24.2	
2006	100	8.2	
2007 (same population, different years)	100	16.7	
Prunus malaheb			
1996	1500	17.9	Godoy and Jordano, 2001
ca. 2005 (same population, different years)	1500	20.3	García et al., 2007
Quercus macrocarpa	100	6	Dow and Ashley, 1996

Several statistical methods were developed to use patterns of allele frequency variation among populations to indirectly estimate the average number of migrants per generation, *Nm,* where *N* is the population size and *m* is the migration rate (Slatkin, 1985a, 1987; Neigel, 1997). Most of these approaches derive *Nm* from overall allelic differentiation, such as that measured by Wright's (1949) F_{ST}. But statistical approaches based on other types of spatial population genetic structure (e.g., "private alleles," Slatkin, 1985b) were also created (reviewed by Neigel, 1997; Ouborg et al., 1999).

For plants alone, hundreds of marker-based studies have described the population genetic structure of various species. Some of these reports estimated *Nm* (examples in Table 4). Furthermore, the authors of reviews on plant gene flow and population genetic structure (e.g., Hamrick, 1987; Govindaraju, 1988a, 1989; Morjan and Rieseberg, 2004) collected those *Nm* estimates and calculated more values from previously reported F_{ST} or other parameters of differentiation.

Gene flow estimates calculated from species-wide population genetic structure are roughly in accord with those from paternity and parentage research for individual populations. Specifically, gene flow estimates for sets of conspecific populations typically sampled miles apart vary over species from very low (*Nm* << 0.5) to very high (*Nm* >> 5.0) (Hamrick et al., 1995) and are often evolutionarily significant, that is, high enough to counteract drift (*Nm* >> 0.5) (e.g., Hamrick, 1987; Govindaraju, 1988a, 1989; Hamrick et al., 1995) (examples in Table 4).

All of the aforementioned population genetic models for estimating *Nm* are based on biologically simple and sometimes unrealistic assumptions (Ellstrand, 1992; Ouborg et al., 1999; Sork et al., 1999; Whitlock and McCauley, 1999). Thus, the accuracy of any individual estimate may be questionable. However, even the strongest critics of the approach find extensive reviews of *Nm* have utility. Whitlock and McCauley (1999) wrote that "comparisons of large groups of species are likely to be more informative, as many of the differences may average out".

As mentioned, such reviews have been conducted for plants, and they have compared large groups of species. For example,

TABLE 4. Some examples of *Nm* calculated from plant population genetic structure

Species	Estimated *Nm*	Reference
Abronia alpine	2.42	Jabis et al., 2011
Blechnum spicant	2.95	Soltis et al., 1988
Botrychium virginianum	0.41	Soltis et al., 1988
Bursera simaruba	3.57	Dunphy and Hamrick, 2007
Cypripedium calceolus	18.0	Brzosko et al., 2002
Dryopteris expansa	0.83	Soltis et al., 1988
Erigeron parishii	0.25	Neel and Ellstrand, 2001
Eriogonum ovalifolium var. *vineum*	3.3	Neel and Ellstrand, 2003
Limonium sinense	0.61	Ding et al., 2013
Panax quinquefolius	1.15	Cruse-Sanders and Hamrick, 2004
Pinus flexilis	11.1	Schuster et al., 1989
Polystichum imbricans	2.20	Soltis et al., 1988
Polystichum minutum	24	Soltis et al., 1988
Stenocereus eruca	0.30	Clark-Tapia et al., 2005
Streptanthus albidus	0.29	Mayer et al., 1994
Streptanthus glandulosus	0.57	Mayer et al., 1994
Streptanthus niger	0.44	Mayer et al., 1994

those reviews report: (1) *Nm* varies with breeding system. Generally, predominantly selfing species have considerably lower migration rates than predominant outcrossers (Hamrick, 1987; Govindaraju, 1988a, b; Hamrick et al., 1995; Morjan and Rieseberg, 2004), and (2) among outcrossers, gene flow varies with pollen vector; wind-pollinated species average more gene flow than animal-pollinated species (Hamrick, 1987; Govindaraju, 1988a, 1989; Hamrick et al., 1995).

Nm estimates based on nuclear loci combine the consequences of both pollen-based and seed-based gene flow. For some species, gene flow by seed can be estimated from the population genetic structure of organelles. In the majority of angiosperm species, plastids are exclusively inherited maternally. In those species, plastid population genetic structure should be determined by gene flow from seeds. The relative contributions of pollen gene flow and seed gene flow can be calculated by comparing plastid population genetic structure to that of nuclear genes. Ennos (1994) was among the first to create a model for comparative analysis. He used the model to calculate the ratio of gene flow by pollen to gene flow by seed for six plant species. In all cases, the ratio was greater than one, that is, gene flow by pollen exceeded gene flow by seed. The smallest calculated ratio (4 : 1) was for wild barley, which is highly self-pollinated; the highest (200 : 1) was for oaks that are known to be wind-pollinated and highly outcrossed. Many more species have been studied since then. Overall, ratios greater than one have been the overwhelming rule (Ouborg et al., 1999; Morjan and Rieseberg, 2004; Petit et al., 2005). For gymnosperms, in which plastids are inherited paternally and mitochondria are inherited maternally, the pollen-dominated seed gene flow ratio is obtained when mitochondrial population genetic structure is used as a proxy for seed-based gene flow (Petit et al., 2005). Interestingly, the observation of pollen-biased gene flow based on plant population genetic structure is congruent with earlier observations from studies of pollen and seed dispersal from sources that showed pollen dispersal curves covered significantly more distance than seed dispersal curves (e.g., Levin and Kerster, 1968).

Reconciling the new data with the old view—The early prediction of the evolutionary insignificance of gene flow in plants based on pollen, seed, and gene dispersal from source plants or populations (e.g., Ehrlich and Raven, 1969; Levin and Kerster, 1974) contrasts with predictions based on the subsequent parentage and population genetic data predicting gene variable, but often considerable, significance (e.g., Hamrick et al., 1995; Ellstrand, 2003b). The predictions are in conflict. But are the data in conflict?

Dispersal studies that have been used to support the Ehrlich–Raven–Levin–Kerster prediction have shown that the number of propagules from an individual or a population falls off rapidly with distance, usually resulting in a leptokurtic curve (e.g., Levin, 1981; Rabinowitz and Rapp, 1981; Handel 1983). Interpretations of those curves often fail to discuss the limitations of such quantitative dispersal studies. But, as Grant (1985, p. 30) observed, "long-range dispersal events are excluded by the methods used in quantitative dispersal studies"; that is, measuring dispersal from a source is only as good as the most distant point sampled. That method often truncates the actual dispersal curve because whether pollen or seed disperse successfully beyond that point remains unknown. Also, the fat-tailed leptokurtosis of the observed distributions predicts more-distant long-distance events than a normal curve exacerbating the

inadequacy of guessing actual gene flow from truncated source dispersal. Additionally, published dispersal curves rarely correct for the fact that sampling circumference increases with distance from a source such that individual sampling sites make up a smaller fraction of the total dispersal at greater distances (but see Klinger et al., 1991; Arriola and Ellstrand, 1996). These three shortcomings all underestimate dispersal. Finally, and perhaps most important, gene flow is essentially defined as successful genetic *immigration* into a population. For the most part, studies that describe the dispersal of propagules or genes out of a source population typically measure *emigration,* often only measuring the departure of pollen or seed from a plant or plants, but not gene flow.

In contrast, parentage and population genetic analyses, despite their limitations, actually estimate gene flow into or among populations. And their results are actually compatible with what would be expected from the leptokurtic dispersal curves. For example, parentage studies often focus on one or a handful of highly isolated, small populations. While the nearest populations might be a kilometer away or more, they might be composed of hundreds, thousands, or tens of thousands of individuals, each producing its own leptokurtic dispersal curve. Although the probability of a single individual siring seed at a kilometer is small, it is not zero. Summing the probabilities over thousands of plants makes the probability of long-distance cross-pollination high.

To illustrate, Ellstrand et al. (1989) created three experimental populations of California wild radish (*R. sativus*), each with 15 plants in a 3×5 array. The three populations were arranged in a triangular fashion at distances of 255, 280, and 400 m. The nearest natural populations, numbering thousands, were more than 650 m from the nearest experimental plant. Paternity analysis identified interpopulation mating rates of 0.2–4.5%. Because the synthetic populations were structured to be fixed for a single allele at two loci, it was possible to obtain a maximum likelihood estimate of gene flow from the surrounding natural populations from alleles that were not present in the experimental populations. That analysis revealed that "all of the gene flow by pollen detected probably originated in the more distant natural populations" (Ellstrand et al., 1989, p. 9046); that is, the three tiny populations, despite being much closer to each other apparently did *not* exchange genes. This kind of size-dependent gene-flow asymmetry is likely to be common (Handel, 1983; Ellstrand and Elam, 1993). These data are consistent with the idea that the combined dispersal curves from a large number of plants can result in gene flow distances and levels that are surprising relative to the prior predictions.

Finally, the general view of evolutionary dynamics of the 1960s and 1970s was ill-suited for assigning gene flow to an evolutionary pantheon. During those decades, natural selection was being enthusiastically adopted by ecologists, systematists, and sociobiologists, opening new doors for "evolutionary" analysis within those fields and leading to remarkable advances. Regrettably, the other forces were largely ignored. Ehrlich and Raven (1969, p. 231) stated the paradigm of the day: "Selection itself is both the primary cohesive and disruptive force in evolution."

The idea of selection and gene flow working in concert to spread beneficial alleles was simply not recognized as a mechanism for evolutionary cohesion. In the late 1970s, I asked Raven to explain why species were largely morphologically uniform over hundreds of miles given gene flow's insignificance. Raven explained that species uniformity over considerable distance was due to stabilizing selection on shared ancient alleles that conferred adaptation over a wide range of environments. Novel

beneficial mutations being spread by gene flow was not an option.

In the 1980s, a low level of attention to the interaction of gene flow and positive selection came almost exclusively from theoretical population geneticists (Slatkin, 1985a, 1987). Empirical evolutionists continued to largely ignore the migration of beneficial alleles. The tipping point occurred around 1995 when analysis of DNA sequence data from the early model organisms (e.g., fruit flies, humans) revealed evidence of "selective sweeps", the rapid dramatic spread of a beneficial allele through a wide portion of a species range (e.g., Nurminsky et al., 1998). Hundreds (at least) of selective sweeps have now been identified. With the acceptance of species cohesion through the periodic spread of beneficial alleles has come the recognition that accepting gene flow as an important evolutionary force does not mean that selection must be dismissed. This new view of gene flow and selection as evolutionary partners has penetrated some, but not all, areas of plant biology with a variety of important implications. For example, as detailed next, the view has profound implications for the nature of plant species.

In other words, the data are not in conflict, only the predictions. Collectively, the plant gene flow research of the 20th century has led to the contemporary view of gene flow. That view differs from the prior views in seeing gene flow as a diverse and dynamic evolutionary force.

THE CONTEMPORARY VIEW OF PLANT GENE FLOW: IDIOSYNCRATIC, BUT OFTEN SIGNIFICANT

The current view of plant gene flow is that it is (1) diverse and idiosyncratic as well as (2) often occurring at levels that are evolutionarily significant at distances of hundreds or thousands of meters. Not only is this view compatible with the data gleaned from the diverse methods discussed, but it has been reinforced by other more recent approaches. For example, analytical models were introduced in the 1990s to estimate gene flow from DNA sequence data (e.g., Hudson et al., 1992). The analysis of gene flow from plant sequence data yields a range and diversity of rates typical of estimates from parentage and population genetic structure (e.g., Hardcastle and Gentry, 2009; Dixon et al., 2010; Heidel et al., 2010; Li et al., 2012). Likewise, GMO biosafety issues associated with crop transgene flow have stimulated numerous experimental studies. These field experiments have sought to determine the rates at which the crops naturally mate with nearby (1 m to as much as many hundreds of meters distant) populations of their cross-compatible wild relatives (often congeners). The experimental rates measured (reviewed in Ellstrand, 2003a, b) vary among species and populations as much as those measured for natural populations reported in Table 2.

***Plant gene flow varies*—**Gene flow in plants can vary tremendously over species, populations, between plants within a population, and even time. The previous Ehrlich–Raven–Levin–Kerster view would not have disputed that gene flow could vary but would have insisted that the primary, and often sole, determinant of plant gene flow would be distance between populations. Scientists studying gene flow today would agree that distance plays *some* role in determining gene flow (say, the difference in gene flow at isolation distances of less than 100 m vs. many hundreds of meters), but not necessarily the *primary* role in explaining gene flow variation. In her review of pollen and

seed dispersal based on microsatellite-based paternity and parentage studies, Ashley (2010, p. 158) summed up this aspect of the current state of affairs nicely:

> "The major surprise coming from microsatellite paternity studies … is *not* that distance is associated with siring success; it usually is… The major surprise is that distance explains only a portion of the variation in mating patterns, often a modest portion, and this holds for both wind- and animal pollinated plants… [T]he assumption of all plant dispersal models is that distance is paramount; it is usually the only parameter considered. The findings reviewed here, however, suggest that models of seed and pollen dispersal based simply on distance will provide poor predictors of plant gene flow… While parentage studies have certainly made the dispersal scene more complex, the emerging paradigm of dispersal is much richer and multifaceted."

Consider the variation in mating rates between roadside populations ("patches") of the perennial vine *Cucurbita foetidissima* Kunth (Kohn and Casper, 1992) detailed in Table 2. This species is gynodioecious, composed of self-compatible, monoecious individuals and female individuals. Furthermore, it is clonally spreading, such that different individuals (ramets) within a population may be the same genetic individual (genet). The interpopulation mating rates reported in that study vary from zero to 64%. The relationship between isolation distance and interpopulation mating is crude. The two most geographically isolated patches (3100 m and 1700 m) were also the most reproductively isolated, and the patch that was closest to another (30 m) was the least reproductively isolated. But the relationship between gene flow and distance is weak for the patches at intermediate isolation distances (80 m to 480 m). In contrast, in that system, the sexuality of individual plants clearly plays a role in frequency of interpopulation mating. In the three populations that were composed of both females and monoecious individuals, the obligate outcrossing females always had a considerably higher fraction of seeds sired by immigrant pollen than the hermaphrodites (cf. Table 2).

Likewise, gene flow can vary considerably over time. For example, Iwaizumi et al. (2013) measured immigrant seed flow into the same natural population of *Pinus densiflora* over 5 yr (Table 3). While the gene flow rate was remarkably uniform for the first 3 yr, the rate fluctuated more than 3-fold (8–25%) over the entire study.

Other factors are known to play an important role in determining gene flow rates. Some of these factors have already been mentioned. As population size increases, the fraction of interpopulation mating tends to decrease. As the relative size of populations exchanging genes diverges, gene flow relationships are expected to be increasingly asymmetric (Handel, 1983). Likewise, selfers tend to have lower gene flow rates than outcrossers. Wind-pollinated species have higher gene flow rates than those with an animal pollen vector.

Interactions between factors also play a role in determining gene flow rate. Goodell et al. (1997) measured gene flow rates between synthetic wild radish populations of varying size (2, 5, 10, and 20 individuals), relatedness (full sibs, half sibs, and unrelated), and varying distances. Because wild radish is self-incompatible, full sibs and half sibs are more likely to be cross-incompatible than distantly related plants. Thus, the authors reasoned that relatedness might be the primary determinant of

gene flow. Seeds from each population were sampled at three times through the season. The nearest conspecifics were at least 90 km from the experimental site. The experimental factors, population size, time of season, and relatedness had significant effects on apparent gene flow rates. Relatedness was the primary determinant of gene flow only for the populations of size two. Many of the interactions (e.g., time × relatedness, time × size, and relatedness × size) also had significant effects on gene flow rates.

To recap, the architects of the Modern Synthesis assumed gene flow was sufficient to unite the populations of a species. The subsequent critics of that view used the extant dispersal data to suggest that natural selection alone unified species. What we now know about plant species suggests that most are structured as semi-isolated meta-populations that exchange genes at different rates that often vary in symmetry and change over time. In the short-term, gene exchange of evolutionary interest typically occurs at the landscape scale (Sork et al., 1999) from 100 to a few thousand meters.

Plant gene flow is often large enough to deserve some respect—In the long-term, whether gene flow is sufficient to serve as evolutionary glue at broader scales depends on its magnitude. In the parentage and population genetic studies reviewed above, plant gene flow rates were frequently found to be evolutionarily significant; i.e., they were high enough to counteract the effects of drift (more than one migrant per generation) and moderate selection (gene flow rates much larger than 1%). For example, it is not unusual for the fraction of successful immigrant pollen to exceed 10% of the seeds set in a small population isolated by a few hundred (and sometimes several hundred) meters from the closest possible male parent.

At the moment, *Ficus sycomorus* L. holds the record. This tree species of trees is pollinated by wind-borne, host-specific fig wasps. A paternity study of a quasi-linear meta-population of this species along a river in the Kalahari Desert reported "a mean distance for confirmed successful pollination events of 88.6 km" (Ahmed et al., 2009, p. 20342). Yes, that's kilometers.

Furthermore, some of the seed dispersal rates and distances are evolutionarily significant. Several natural populations summarized in Table 3 have immigrant seed gene flow rates that exceed 5%, sometimes at eyebrow-raising distances. Those long and fat leptokurtic tails not only predict occasionally evolutionarily significant gene flow, but also ecologically significant colonization. In fact, fat-tailed curves are necessary to explain the rapid multithousand-kilometer post-Pleistocene migrations of hundreds of plant species (e.g., Clark, 1998).

IF THE CONTEMPORARY VIEW OF PLANT GENE FLOW IS CORRECT, WHAT ARE SOME CONSEQUENCES?

Gene flow happens. Not necessarily for all plant species and plant populations, but for many. Not always at evolutionarily significant rates, but often enough to deserve recognition. Next, I consider what the contemporary view of plant gene flow means for plant evolutionary biology and for applied plant biology. The examples are far from exhaustive but reveal that gene flow biology involves more than just measuring rates and distances.

Consequences for plant evolutionary biology—If gene flow in plants cannot be ignored, then two obvious consequences should follow: (1) Gene flow should, at least occasionally, act as an evolutionary glue that unites species. (2) The ability to receive gene flow—despite the fact that it is a trait dependent on an interaction (such as "chemical defense")—should be able to evolve.

Gene flow as evolutionary glue?—The mid-20th century view of evolutionarily insignificant gene flow implies that the biological species concept has no validity because what might be conceived of as "species" are united genetically only by shared phylogeny and are united morphologically only by stabilizing selection. Not surprisingly, the reality of the biological species concept has been challenged, bolstered by the mid-20th century view (Levin, 1979; Mishler, 1999, reviewed by Rieseberg et al., 2006).

At the turn of the 21st century, Rieseberg and colleagues reasoned that species cohesiveness should be based on connectivity due to the periodic spread of globally beneficial alleles. They reassessed the biological species concept based on the newest data on gene flow and fitness differentials (Rieseberg and Burke, 2001; Morjan and Rieseberg, 2004). Specifically, they asked: (1) Are gene flow rates and distances sufficient to speed the spread of beneficial alleles generation by generation? (2) Are the selective coefficients for different alleles of sufficient magnitude to collaborate with gene flow? With regard to gene flow, they reviewed both the parentage and population genetic data for hundreds of species, finding that for the vast majority of species gene flow was sufficient to move neutral alleles across populations and therefore would be more than sufficient to speed the spread of strongly advantageous alleles (Rieseberg and Burke, 2001; Morjan and Rieseberg, 2004). With regard to selection, they again surveyed hundreds of species. They found that "crude estimates on the strength of selection on phenotypic traits and effect sizes of quantitative trait loci (QTL) suggest that selection coefficients for leading QTL underlying phenotypic traits may be high enough to permit their rapid spread across populations" (Morjan and Rieseberg, 2004, p. 1341) while allowing for some local adaptation (Rieseberg and Burke, 2001). In sum, gene flow can lend cohesion through the creative activity of spreading globally advantageous alleles among reproductively compatible populations. Thus, the biological species concept works well for species that are outcrossed or have a mixed mating system, but not necessarily for lineages that have restricted seed dispersal and are highly selfed or highly apomictic.

Gene flow as an evolving trait—Consideration of the evolution of gene flow is hardly novel. The evolution of reproductive isolation—that is, reduced gene flow—has been a frequent topic for a diverse set of research endeavors in plant evolution—including the biological species concept, speciation, local adaptation, character displacement, and reinforcement (e.g., Widmer et al., 2009). Plant traits at the individual level, ranging from floral morphology to cross-incompatibility to phenology, are known to vary in plant populations and that variation is known to frequently have a substantial genetic basis (e.g., Young et al., 1994; Weis and Kossler, 2004; Kermicle and Evans, 2010). A recent burst of research measuring the strength of different isolation barriers between related plant species has revealed that the barriers between good species within a genus typically collectively exceed 99% isolation (e.g., Lowry et al., 2008).

A few facts are worth noting at this point. First, if gene flow rates are insignificant, then evolution of local adaptation could easily occur *without* evolution of reproductive isolation. Second, if gene flow rates are significant (ca. 5% or greater), then selection for local adaptation must be *very strong* (selective coefficient >> 5%) to counteract gene flow. When selection is strong for local adaptation in the face of countervailing gene flow, selection for reproductive isolation may also occur.

Let's consider a classic case of the evolution of joint local adaptation and reproductive isolation. In Great Britain, numerous ancient metal mines with spoiled soils highly toxic to most plants have been colonized by a few species from adjacent populations only a few meters from their edges. The colonists are tolerant of the spoiled soils; the adjacent source populations are not. The evolution of heavy metal tolerance of plants at these sites has been the subject of intense study (e.g., Antonovics et al., 1971; Shaw, 1989). The selection pressure is intense, estimated at >99% (Antonovics, 1976). In a series of studies, Antonovics and McNeilly asked whether and how reproductive isolation might have evolved in these highly outcrossing species in parallel with adaptation to toxic soil in the face of considerable gene flow from closely adjacent plants.

They found that reproductive isolation had indeed evolved, resulting in reduced gene flow into heavy metal tolerant populations. McNeilly and Antonovics (1968) studied adjacent populations of tolerant and nontolerant populations of *Agrostis tenuis* Sibth. and *Anthoxantum odoratum* L. They found that mine (tolerant) populations of both species flowered about a week earlier than the adjacent nontolerant populations; flowering time is well known to be genetically controlled. Antonovics (1968b) also asked whether the mine plants had evolved self-fertility, which is often correlated with reduced gene flow. *Agrostis tenuis* and *Anthoxantum odoratum* are typically obligately outcrossing self-incompatible species. He found populations on mine soils had much higher levels of genetically based self-fertility than adjacent nontolerant populations (Antonovics, 1968b). Antonovics (2006) revisited one of the populations 40 yr later and found that reproductive isolation had persisted in these plants whose half-life is about 2 yr. He calculated an "isolation index" of 0.43 between the tolerant and nontolerant populations; that is, "even if the populations were intermixed, there would be about 40% less mating between tolerant and nontolerant populations than within those populations" (Antonovics, 2006, p. 35).

Descriptive and experimental studies of the evolution of reproductive isolation in plants abound. Studies of the evolution of reduced reproductive isolation are curiously rare. What would evolution of reduced reproductive barriers look like? One example would be the evolution of a lineage that acts as a bridge between parental taxa. Are there any examples of hybrid lineages that have evolved to reproductively unite their reproductively isolated parents? The best-studied candidates may be recently evolved invasives and weeds that are descendants of interspecific hybrids (Schierenbeck and Ellstrand, 2009), specifically, the best candidates are the hybrid-derived lineages that have not been stabilized by clonal reproduction or allopolyploidy. A handful of these so-called "coalescent complexes" have been recognized (tables 1 and 2 in Schierenbeck and Ellstrand, 2009). Some of these complexes may be fully cross-compatible with their ancestral parents.

One of the best examples appears to be what is called "California wild radish" (Hegde et al., 2006). The genus *Raphanus* is native to the Old World. Cultivated radish, *R. sativus*, and its

weedy relative, *R. raphanistrum* L. naturally hybridize when they co-occur, often forming transient, localized hybrid swarms (e.g., Stace, 1975). However, in California, bilateral introgression between the two introduced taxa (Ridley et al., 2008) over the past hundred years or so has been so extensive that the two separate species have coalesced into a widespread set of hybrid-derived populations, as confirmed by both morphological and molecular analyses. The populations are in Hard–Weinberg equilibrium and have a number of traits that are relatively uniform. At the same time, the coalescent complex is not simply a giant hybrid swarm; it is significantly different from both parents in certain traits (Hegde et al., 2006). The historical record shows a gradual spread of hybrid-derived populations that eventually replaced the pure populations of the ancestral taxa in California (Frost, 1923, Panetsos and Baker, 1967; Hegde et al., 2006). A common garden study showed that the flowering time of the California populations is intermediate with and overlaps with both parents (Hegde et al., 2006). Field experiments have also shown that California wild radish easily spontaneously hybridizes with its domesticated parent, resulting in fertile and vigorous progeny (Klinger et al., 1991; Klinger and Ellstrand, 1994). While similar data for spontaneous hybridization between California wild radish and its other parent have not yet been reported, the current data are compatible with the evolution of a lineage that has relaxed reproductive barriers between its parents so that it can forage for useful alleles that have permitted it to drive both parents to extinction in California. Perhaps other coalescent invasive and weedy lineages are doing the same elsewhere. In any case, study of the evolution of reduced isolation represents a nearly virgin area of plant evolutionary research.

Applied consequences—The range of human concerns relating to the genetic composition of plant populations is staggering. Natural gene flow and human-mediated gene flow are a component of many of these concerns. As discussed, breeders and seed-producers are concerned with maintaining the genetic purity of seed at a certain level and consequently preventing genetic contamination by accidental pollen- and seed-based gene flow. With regard to plant conservation, significant gene flow changes can alter the genetic composition of the populations of endangered species for better or worse (Ellstrand and Elam, 1993). Consideration of gene flow can play a role in the success of activities allied with plant conservation such as restoration, genetic rescue, and assisted migration (e.g., Montalvo and Ellstrand, 2000, 2001). Also, intraspecific and interspecific gene flow has been recognized to sometimes act as a stimulus for the evolution of weediness and invasiveness (Barrett, 1983; Schierenbeck and Ellstrand, 2009). With the rise of the plant biotechnology industry, consideration of gene flow plays a role in the security of intellectual property (Ellstrand, 1989) and trade and coexistence issues (Ellstrand, 2012), and the analysis of certain biosafety risks (National Research Council, 2002).

A thorough discussion of the role of gene flow in applied plant biology is beyond the scope of this article. The topic will be covered in detail in a review now underway (N. C. Ellstrand, unpublished). A few of the current prominent applied issues are covered briefly here.

Gene flow as a vehicle for the unintended movement of engineered genes has generated considerable discussion over a wide diverse group of scientists for decades (e.g., Colwell et al., 1985; Goodman and Newell, 1985; Ellstrand, 2001; National Research Council, 2002, 2004; Stewart et al., 2003; Andersson

and de Vicente, 2010; Kwit et al., 2011). The major focus has been the role of spontaneous gene flow associated with certain environmental biosafety hazards.

Spontaneous gene flow from traditionally improved crops to their free-living relatives has received considerable research attention and serves as a model for predicting whether transgene flow will occur. When most crops and their cross-compatible wild or weedy relatives (often conspecifics) occur close enough to cross-pollinate, natural hybridization is not unusual (Ellstrand, 2003a; Andersson and de Vicente, 2010). And just like natural species, the gene flow rates vary considerably over the species and the populations involved (Ellstrand, 2003 a, b).

Information regarding crop allele introgression into wild and weedy populations is more limited but increasing. The natural introgression of crop alleles into natural populations is known for several crop species. Notable examples include sunflower, sugarbeet, and Asian domesticated rice (Ellstrand et al., 2013). Again, the patterns of gene flow are idiosyncratic among the species and populations studied.

One long-term experimental study revealed that even individual alleles can have different patterns of introgression. Snow and colleagues (Campbell et al., 2006; Snow et al., 2010) studied the consequences of domesticated radish (*R. sativus*) allele introgression into naturalized populations of the wild species *R. raphanistrum*. Under natural field conditions, they created four populations of crop–wild F1s, allowing these annual plants to evolve for more than a decade. They monitored a handful of unlinked crop-specific alleles; each allele showed a different evolutionary trajectory. One crop trait, a dominant white-flower-color allele, remained present in the introgressed populations 14 yr after the experiment's start.

Gene flow, being a common natural phenomenon, is not a problem in itself. Indeed, more than 20 well-documented incidents of crop transgenes out-of-place (sometimes called "escape") have occurred since the first field release of an engineered crop in 1987 (Ellstrand, 2012). Some of these events likely occurred by unintended pollen exchange and others by seed mixing (anthropogenic or otherwise). Such gene flow is not a hazard in itself. With regards to risk assessment, gene flow can act as the "exposure" component of the risk equation (National Research Council, 1995) for specific environmental hazards.

The appropriate regulatory approach for transgene flow risk assessment was succinctly stated by Val Giddings, then Vice President of the Biotechnology Industry Organization, in a 2001 listserve post. "As a regulator working for the US Department of Agriculture, … I always assumed that if the probability of gene flow between a transgenic and a wild relative was not zero, then it was taken for risk assessment purposes to be one. Then and only then can one really focus on the relevant question, which is not 'will there be gene flow?' but rather 'what consequences might be expected in the event of gene flow?'" (AgBioWorld, 2001). Thus, a crude knowledge of whether gene flow will occur is critical for risk assessment, but detailed studies are not. Given the great variation in gene flow now known from experimental and description studies, Giddings' guidance to quickly move to start characterizing undesirable consequences ("hazards" in the parlance of risk assessment [National Research Council, 1995]) is wise.

The two potential consequences of transgene flow have generated the most attention: (1) the evolution of increased weediness or invasiveness and (2) increased extinction probability. Both of these are also of more general concern, and they will be

discussed in the context of transgene flow as well as more broadly below.

"The sexual transfer of genes to weedy species to create a more persistent weed is probably the greatest environmental risk of planting a new variety of crop species" (Goodman and Newell, 1985, p. 51). Note that this statement by two Calgene scientists is not restricted to transgenic crops, but applies to any new crop variety, transgenic or not. Gene flow from a crop to a wild or weedy (often conspecific) relative has resulted in the evolution of at least eight significant weedy and invasive lineages (Ellstrand et al., 2010). The word "superweed" to describe such nasty hybrid lineages was coined (Anderson, 1949) long before contemporary genetic engineering could have been imagined.

In fact, the problem is much more general. A crop parent is not necessary for gene flow to act with selection to build plants that create nightmares for humans. Schierenbeck and Ellstrand (2009) listed over three dozen novel weedy or invasive plant taxa or lineages that were descendants of inter- or intrataxon hybrids. Several more examples have accumulated since then (e.g., Keller and Taylor, 2010; Paul et al., 2010). Even though gene flow is not necessarily a prerequisite for the evolution of invasiveness or weediness (see examples of "endoferality" in Ellstrand et al., 2010), the hazard of the evolution of problematic plants not only warrants consideration in transgenic risk assessment (Ellstrand, 2001) but is a hazard that transcends transgenics. The ability to predict whether an introgressed crop gene, transgenic or not, will result in a more problematic plant is rarely straightforward and remains an issue worthy of focused study.

The other concern associated with transgene flow, increased risk of extinction, also transcends transgenics. Intertaxon (within-species or between-species) hybridization has been recognized as a potential conservation problem for sensitive plant populations for decades (e.g., Ratcliffe, 1973; Ellstrand and Elam, 1993). The introgression of alleles from one taxon into one that is endangered can have two different negative, and not necessarily mutually exclusive, consequences. The first is genetic swamping (also known as "genetic assimilation"). Ratcliffe (1973, p. 21) observed "species may be disappearing through introgression of a rare plant with a more common relative to produce hybrid swarms in which the characters of the rare species are finally swamped." A few plant examples are known, mostly involving domesticates mating with close wild relatives (Ellstrand, 2003a). For example, genetically pure individuals of coconut's (*Cocos nucifera* L.) wild ancestor apparently do not exist; no free-living coconut tree is known that does not have a few genetically based characters associated with the domesticate (Ellstrand, 2003a). Likewise, the hybrid-derived lineage "California wild radish" detailed earlier, appears to have extirpated its parents in California by assimilating them into the new lineage (Hegde et al., 2006).

The other negative consequence of gene flow is "outbreeding depression", mating between taxa or between populations producing progeny with reduced fitness compared with progeny from within-population mating. Reduced fitness in interspecies hybrids is so well known that the many cases of increased fitness in hybrids are often ignored (Arnold, 1997). A relevant example involves the case of natural gene flow from *Oryza japonica* varieties of rice with the endemic wild rice *O. rufipogon* Griff. subsp. *formosana* in Taiwan. The decreased seed set and pollen fertility of the frequently produced hybrid progeny apparently contributed to the endemic's extinction in the wild (Kiang et al., 1979; Oka, 1992). But even gene flow among well-differentiated populations of the *same* taxon can result in outbreeding depression (Waser, 1993).

Some modeling of extinction via gene flow has already been accomplished (e.g., Wolf et al., 2001). Given that populations already at risk for extinction are often small, any substantial increase in gene flow from an offending population can increase the risk of extinction, by genetic swamping, outbreeding depression, or both. Transgenes per se do not pose a problem, but any substantial increase of alien alleles, transgenic or not, will lead to increased swamping. Any substantial increase of alleles that decrease fitness or otherwise affect population sustainability (again, transgenic or not) will challenge the future sustainability of the sink population (e.g., Muir and Howard, 1999).

A LOOK TO THE FUTURE

Research on plant gene flow is alive and growing in the 21st century. Gene flow is beginning to get the respect in evolutionary biology it deserves. This revival for gene flow goes beyond simple documentation that it does exist.

Gene flow is a component of the new integrative biology (e.g., Pannell and Fields, 2013). Genomic data with bioinformatic analysis reveals that gene flow and selection can work well in cooperation. Work on such adaptive introgression has begun in earnest bringing plant evolution research full circle to Anderson and Stebbins' vision of advantageous alleles flowing from one species to another (Rieseberg and Yakimowski, in press). Whitney et al. (2006, 2010) took advantage of the fact that the genome of cultivated sunflower has been thoroughly sampled for QTL markers. They studied *Helianthus annuus* subsp. *texanus* Heiser, the hybrid derivative of wild *H. annuus* subsp. *annuus* L. and *H. debilis* Nutt. They grew these three taxa, synthetic hybrids, and backcrosses in two common gardens and measured how fitness covaried with herbivore pressure as well as QTL-labeled ecophysiological, phenological, and architectural traits. They were able to identify the adaptive introgression of a number of specific abiotic and biotic traits into *H. annuus* subsp. *texanus*.

Such research has not been restricted to interspecific gene flow. Hufford et al. (2013) investigated the role of gene flow in the origin of the adaptive differences between lowland and highland maize (*Zea mays* subsp. *mays*) landraces in central Mexico. Maize is known to have been domesticated from a wild lowland subspecies (*Z. mays* subsp. *parviglumis* Iltis & Doebley). The presence of a high-elevation subspecies [*Z. mays* subsp. *mexicana* (Schrad.) Iltis; henceforth, "mexicana"] gave rise to the hypothesis that gene flow from the latter delivered adaptive alleles permitting the successful evolution of maize adapted to highland conditions. Comparing nine sympatric maize–mexicana paired populations with appropriate reference allopatric populations, Hufford et al. (2013) assessed patterns of genome-wide introgression in this system using over 39 000 single nucleotide polymorphisms. They found portions of the genomes of both maize and mexicana to be resistant to introgression, notably in the vicinity of known cross-incompatibility and domestication loci. But they also detected evidence of bilateral introgression elsewhere. Coupling further genome analysis with growth chamber experiments, they found evidence of adaptive introgression from mexicana to highland maize. Specifically, two traits previously assigned a putative adaptive role for highland maize, anthocyanin content and leaf microhairs, were associated

with the highly introgressed regions of the highland maize landraces. Interestingly, little evidence was found for adaptive gene flow in the other direction, from maize to mexicana.

If new techniques are opening doors for gene flow research, the same can be said for new questions based on the contemporary view of gene flow. Research questions associated with gene flow are no longer hampered by the premature reports of its demise. This article has already hinted at numerous relatively virgin areas for both basic and applied research. One more is offered, gene flow that is nonrandom with respect to the genotypes of the dispersing propagules.

Nonrandom gene flow was recently explored by two animal biologists as an "underappreciated force in evolution and ecology" (Edelaar and Bolnick, 2012), but its potential significance in plants is equally substantial. In plants with animal pollen vectors, individual pollination events probably never involve a random collection of pollen—and that would be a certainty for species that produce pollinia. Likewise, multiseeded dispersal units such as fruits represent half-sib or full-sib families. Thus, the dispersal of seed families as a unit represents "kin structured migration", which has received attention in human-gene-flow research, but has rarely been considered for plants (Levin and Fix, 1989). As noted earlier, kin structure in small, self-incompatible populations that could have been founded by a single fruit can have a significant impact on subsequent mating patterns between populations (Goodell et al., 1997). Experimental work has also shown that whether kin structure is present in a small self-incompatible population can have a subsequent impact on plant fitness because nearby individuals may be cross-incompatible (Elam et al., 2007).

The foregoing topics are only a taste of the future of gene flow research. Global change is one of many factors stimulating new applied gene flow research questions. The reality of moving climates has brought attendant attention to the natural and human-mediated movement of alleles to mitigate climate change (e.g., Jump and Penuelas, 2005; Aitken and Whitlock, 2013). Gene flow research is unlikely to go out of style in the foreseeable future.

CONCLUSIONS

Is gene flow the most important evolutionary force in plants? That's a silly question!

Concentrating on any single evolutionary force ignores the richness of the evolutionary process. Evolution is fundamentally the product of more than a single factor. Beyond the four classic "forces", evolution also depends on various constraints such as history, demography, development, organismal structure, genomic structure, and environment (e.g., Antonovics, 1976; Ellstrand, 1983; Gould and Lewontin, 1979). The point here is that none of these should be stereotyped without good reason.

Clearly, gene flow is neither universally ubiquitous nor universally absent in plants. Its magnitude and quality in any particular dynamic, however, may determine the evolutionary pathway that unfolds. Although some scientists may still give gene flow short shrift in their evolutionary discussions (e.g., Zimmer 2010), the majority of contemporary plant evolutionists have come to embrace gene flow's importance. Gene flow is an important component of both basic and applied plant evolutionary biology that deserves the increasing respect that it is at last beginning to receive.

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