Introduction

The California grassland represents one of the most thoroughly invaded ecosystems on the planet (Mack 1989) and so perhaps it is not surprising that much of the early work on the population genetics of component species focused on invasive species, in particular, annual grasses. Much of the early work by Subodh Jain and Robert Allard at UC-Davis represented direct offshoots of their more agronomic work on long term evolutionary dynamics of barley mixtures. In an effort to understand how selection may alter population genetic structure within inbreeding species (Jain and Allard 1960, Allard and Jain 1962), they expanded their research programs on the evolution and genetics of selfing species to include other species of Mediterranean annual grasses such as wild oats (Avena spp.) or annual fescues (Vulpia spp.). The grassland species they focused on were annuals that were easy to germinate and grow and demonstrated interesting and suggestive patterns of genetic variation on both a regional and local level. At UC-Berkeley, Herbert Baker was quick to recognize that many of the invasive species that were the scorn of most California botanists actually represented fascinating model systems for understanding the dynamics of colonizing species and the speed at which evolutionary change may occur. In fact, the symposium volume “The Genetics of Colonizing Species” co-edited by Baker (Baker and Stebbins 1965) remains one of the best syntheses of population ecology and population genetics; many of the areas in plant
population biology that represent current “hot topics” can trace their roots to this truly seminal volume.

Interest in the population biology and ecological genetics of native species within California grasslands has grown. In particular, highly variable success in the attempts to restore native components of California grassland ecosystems (Stromberg and Wirka, this volume) has generated a real need to understand patterns of local adaptation. The restoration practitioner’s perennial question of “How local is local?” is a difficult one to answer and represents a situation where information needed for practical application and policy currently exceeds our basic scientific knowledge. A good answer to this question requires better knowledge in a number of areas such as historical and current variation in gene flow, scales of adaptation, spatial and temporal variation in the form and intensity of selection, and the relative role of biotic and abiotic selective agents (Box 1).

Unfortunately, relatively few studies of native species combine modern molecular techniques to estimate gene flow with field based approaches, such as common gardens and reciprocal transplant experiments, to estimate local adaptation. This combined approach is necessary to understand the relative strengths of gene flow and selection in the formation of adapted ecotypes.

Although much remains to be done to understand fundamental processes in the population genetics of California grassland species, the work we review in this chapter has provided many important insights into processes of microevolutionary change in plant populations. Basic population genetics concepts ranging from co-adapted gene complexes to evolution of phenotypic plasticity were initially proposed and developed by researchers working in the California grasslands. Ironically, the potential for restoring
native species within the system may depend on these insights gained by studying tractable “model” invasive members of California’s grasslands.

**Genetics of Colonization**

**The origins of invasive species and the role of genetic bottlenecks**

It is almost axiomatic that the genetic diversity of an entire species is not present within the populations invading a new range (see Cox 2004 for examples). Typically, the initial introduction event creates a significant genetic bottleneck; genetic variation of the introduced populations is but a small subsample of the genetic variation in the species’ home range. The amount of genetic variation within an introduced population may determine its capacity to adapt and spread within its new range so the study of genetic bottlenecks is of fundamental interest and has implications for management strategies. The breeding system of the species is often a prime determinant of the severity of this bottleneck in the initial colonizers. Outcrossers would be expected to exhibit less-pronounced bottlenecks because outcrossing species typically exhibit higher levels of individual heterozygosity as well as greater levels of within-population genetic variation than inbreeders. The severity of the bottleneck can also be greatly reduced if multiple introductions occur during the initial establishment phase of the invader. In fact, depending on their origins, multiple introductions can radically transform the genetic architecture of the species in its new range. For example, if individuals from genetically distinct sub-populations in the home range are combined in the invading population, genetic variation within the colonizing population may actually be greater than the
genetic variation found within any of the source populations in the home range (Cox 2004).

Identifying the origins of invaders is also important for deducing transport vectors and assessing the potential for pre-adaptation in the introduced populations. Colonizers from climatic zones or habitat types similar to the site of introduction are expected to be more successful than genotypes that have not evolved under similar conditions (e.g. pre-adaptation). Identification of invader origins may also be of great practical importance for developing biocontrol programs for the invasive species (DeBach and Rosen 1991).

The development of allozyme markers in the late 1960’s (Lewontin and Hubby 1966) facilitated efforts of California researchers to trace the origins and colonization dynamics of introduced species in our grasslands. In particular, patterns of genetic variation in *Avena barbata* (slender wild oats) were examined in both California and Mediterranean populations. Allozyme markers and inherited morphological traits were used to understand the relative importance of chance colonization, genetic drift, and selection in changing genetic structure between home range and new range populations of *A. barbata*. The earliest study by Clegg and Allard (1972) focused on differences in the regional patterns of genetic variation between California and Mediterranean populations. They sought to test if differences in genetic structure were random in nature (i.e. caused by chance colonization and genetic drift) or non-random and correlated with environmental parameters (i.e. the result of selection). Using rather sparse sampling from a very large putative home range, the authors concluded that California populations probably represented a random sample of the genetic variation from the entire home range. Clegg and Allard (1972) also concluded that selection along climatic gradients, not
random drift, created the regional pattern in California: patterns of regional
differentiation in California between drier/warmer locations (the “xeric genotype”) and
wetter/cooler locations (the “mesic genotype”) were non-random subsets of the original
genetic sample from the home range. They further presented the more general (and more
controversial) conclusion that allozyme variation in this system was not neutral to
selection. In a later extension of this idea, de la Vega (1996) argued for the non-
eutrality of allozymes (as well as other molecular markers) and used examples from the
*A. barbata* system as evidence that molecular polymorphisms can be involved in
processes of adaptation.

A more thorough genetic survey of both California and Mediterranean *A. barbata*
populations was conducted by Garcia et al. (1989) to further circumscribe the source of
California populations. In contrast to Clegg and Allard (1972), they found that California
*A. barbata* did not comprise a random sample of its home range in the general
Mediterranean region. Rather it appeared that California *A. barbata* populations
originated from southwestern Spain (supported by Perez et al. 1991); southwest Spain
was a region of intense trade activity with the New World around the time the species
was introduced to California. Garcia et al. (1989) argued that the climate of southwestern
Spain is similar to that of California so that introduced genotypes may have been pre-
adapted to the general environmental conditions present in their new range. They then
compared genetic structure in some detail between populations in Spain and California by
examining variation in alleles as well as single-locus and multi-locus genotypes. Their
examination of allelic variation indicated that a significant bottleneck occurred during the
introduction of *A. barbata* because California populations contain only a small fraction of
the infrequent or rare alleles present in Spain. This study, and subsequent work by de la Vega et al. (1991), emphasized that although Spanish and California gene pools are very similar in allelic frequencies, there are pronounced differences in the way the alleles are associated with each other in forming multilocus genotypes. In particular, the “mesic” and “xeric” multi-locus genotypes that dominate California populations are not present in Spain. Both Garcia et al (1989) and de la Vega et al. (1991) considered this reorganization of alleles to be strong evidence for the role of selection in creating regional and local genetic structure in *A. barbata* as it colonized and spread throughout California.

Bottlenecks may be especially severe in inbreeding species introduced more recently than long term residents such as *A. barbata*. Multiple introductions, that might reduce bottleneck effects, are less likely to have occurred in a very recently introduced species. This expectation appears to be supported by recent research on the genetic diversity and origins of *Aegilops triuncialis* (barbed goatgrass). *Aegilops triuncialis* was introduced to California in the early 1900’s from a home range spanning the entire Mediterranean and extending into the Middle East. *Aegilops triuncialis* has recently begun to spread rapidly throughout northern grasslands. Using a series of chloroplast and nuclear molecular markers, Meimberg et al (in press) examined genetic diversity in several California populations relative to home range populations. Because *Ae. triuncialis* is used in wheat breeding, an exceptionally extensive collection of germplasm from the home range was possible. From this survey it appears that *Ae. triuncialis* underwent a major bottleneck during its colonization of California rangelands. Meimberg et al. (in press) could identify only two distinct genotypes in California that
correspond roughly to Coast Range and Sierra Foothill colonization events. The authors speculated that the capacity of *Ae. triuncialis* to spread may rely on a combination of phenotypic plasticity as well as mutation in genes underlying quantitative traits.

Theory predicts that genetic bottlenecks should be less pronounced in outcrossed species (Conner and Hartl 2004). Although empirical data on invasive outcrossers is relatively scarce, the studies that are available generally support the predictions. A study by Wu and Jain (1980) on coastal California populations of the outcrossing perennial grass *Anthoxanthum odoratum* (vernal grass) examined variation in three allozyme loci. They found significant polymorphism for all three enzyme systems and average heterozygosity for the three loci was relatively high (0.24 – 0.43). In studies on *Trifolium hirtum* (rose clover), Molina-Freaner and Jain (1992) reported that there was little indication of a reduction in genetic variation of California populations relative to home range populations in Turkey. In fact, they found that relative genetic variation within California populations was higher than in Turkey populations and that the number of multi-locus genotypes was also higher in California. Because this species was purposefully introduced as a forage species for range improvement (Love and Sumner 1952), it is likely that there was a concerted effort to incorporate high genetic diversity in the initial plantings. In addition to a high genetic diversity of the initial introductions, Molina-Freaner and Jain (1992) also suggested that increased outcrossing rates that foster gene flow among different sub-populations may reduce genetic bottlenecks during future colonization events. In a rare test of the importance of genetic variation in colonization success, Martins and Jain (1979) monitored the demography of experimentally established roadside colonies of *T. hirtum* over a seven year period. Although genetic
polymorphism *per se* was not a significant determinant of colony persistence, they found that certain rose clover genotypes were more likely to germinate and successfully reproduce in some years than others. This significant year by genotype interaction in fitness suggested that different genotypes are better adapted to certain types of years. The retention of an array of “year specialist” genotypes in the seed bank may facilitate colony survival and spread and may thus represent a selective response to a growth environment that is highly variable from one year to the next.

**Genetic variation in peripheral populations – Genetic drift and founder effects**

The inherently patchy distribution of many plant populations within California grasslands has led many researchers to speculate on how metapopulation processes of colonization and extinction might increase the likelihood of founder effects and genetic drift. In annual species, where the dynamics of colonization and extinction are especially pronounced, determining the relative importance of selection versus random genetic drift has proven difficult. One strategy has been to examine populations that, because of their relative isolation or peripheral location, would be more likely affected by founder effects and thus express differentiation resulting from random genetic drift. In an early study of populations of *Avena fatua* (wild oats) within prune orchards, Jain and Rai (1974) capitalized on the agricultural practices within the orchards to examine the effects of population subdivision, extinction, and recolonization on genetic polymorphism in both morphological traits and isozyme markers. Although not conducted within California grasslands, the study is worth noting because of the relevance of its results to a more general understanding of how metapopulation dynamics can influence patterns of genetic
variation in grassland annuals. Cultural practices within the orchards reduced *A. fatua* populations to small colonies clustered around the base of each tree that were isolated from other such colonies in the orchard. Two orchards were studied that, because of differences in tillage operations, were distinct in terms of relative colony size; the average census size of *A. fatua* populations in one of the orchards were about twice that of populations in the other orchard. Citing data on restricted seed dispersal, inbreeding, and low seedling survival between colonies, the authors argued that this system closely approximated Wright’s model of a large metapopulation subdivided into a series of isolated small populations. Overall, the authors found that there was a large degree of genetic differentiation among colonies with many colonies monomorphic for genetic markers. As might be expected for a series of small isolated populations, this mosaic of genetic differentiation was random in nature and apparently dominated by drift effects. The authors found no significant variation among genotypes along clinal gradients within the orchard suggesting that selection was not important and that drift effects predominated in structuring genetic spatial patterns. In addition, measurements of fecundity among individuals within the colonies indicated that high reproductive variance within the colonies significantly reduced effective population size; another factor that would increase the likelihood of drift effects. Although these studies were conducted in prune orchards, it is not difficult to imagine that drift could play a similar dominant role in more natural grassland populations that are patchily distributed, isolated by low gene flow, and undergo localized extinction and colonization.

Further examination of the potential importance of drift effects during founder events was undertaken by Jain et al. (1980) in their comparison of genetic variation in
morphological traits and allozyme markers between central and isolated roadside populations of *A. barbata*. In general, their results supported the importance of drift effects in creating spatial differentiation between large central populations and smaller, peripheral roadside colonies of *A. barbata*. Roadside populations were more likely to be monomorphic for genetic markers and exhibited much stronger differentiation among populations. Both of these results suggest the importance of founder effects. The authors, citing evidence presented by Endler (1977), further noted that genetic effects of initial founding events can persist for many generations even in the face of substantial gene flow. The persistent influence of founding events on genetic variation was further demonstrated in a later study by Rai and Jain (1982) on the effects of gene flow on spatial genetic variation in *A. barbata*. In this study, natural populations of *A. barbata* at four different grassland sites did not suggest any bottlenecks in population size, and allelic frequencies remained stable over a three year period. Overall, these studies on the importance of random processes such as genetic drift and founder effects caution against the assumption that selection is the predominant force in forming the genetic architecture of many grassland species. Particularly for inbreeding annuals undergoing repeated extinction and colonization, the combination of restricted gene flow and small effective population size would suggest that genetic drift is an important “player” in the evolution of grassland metapopulations.

Inbreeding depression can be a cryptic barrier to successful colonization. In particular, colonizing plants that are outcrossers can suffer from inbreeding depression due to lack of mating partners. Measuring inbreeding depression includes not only an examination of seed set within inbred plants, but following the progeny of these plants
through their life cycle and comparing them to outcrossed lines. In a study comparing seed performance of selfed and outcrossed *Trifolium hirtum* (rose clover) (Molina-Freaner and Jain 1993), germination between the two inbred and outcrossed plants was the same, but survival was significantly less for seed produced from the selfed plants. Similar results were found for *Delphinium hesperium* (western, or coast, larkspur), *D. recurvatum* (valley, or recurved, larkspur) and *D. gypsophilum* (gypsum-loving larkspur): selfed plants had seed set as high as outcrossed plants, but seeds produced from selfing had lower germination rates (Epling and Lewis 1959). In studies within the *Limnanthes* (meadowfoam) genus, it appears that the species *L. floccosa* (woolly meadowfoam) evolved an inbreeding mating system in addition to considerable phenotypic plasticity in order to be a more successful colonizer than its congenerics (Jain 1981).

While it might be expected that successful colonizers are inbreeders, evolutionary trajectories do not always operate that way: *Lupinus succulentus* (arroyo lupine) is an outcrossing species with heterozygote advantage, as opposed to its non-colonizing relative *Lupinus nanus* (no common name) which has similar outcrossing rates and similar amounts of genetic variability (Harding and Barnes 1977). Outcrossing species are subject to genetic drift: several colonial populations of *L. succulentus* were found to be fixed for some genetic markers (Harding and Barnes 1977). When the colonization environment is variable, genetically diverse colonizers are more successful, as variable environments facilitate the maintenance of heterozygote advantage and an outcrossing breeding system (Lewontin 1965).

**Breeding Systems**
Gene flow and breeding systems

Gene flow, or the migration of genotypes among populations, is the primary microevolutionary force of sufficient strength to counter the effects of natural selection. The balance between gene flow and selection is important to understand because the likelihood of local adaptation occurring depends on the relative effects of selection and gene flow (Kirkpatrick and Barton 1997). Surveys of population structure (e.g., McKay and Latta 2002, Kittelson and Maron 2001) have indicated that even under significant rates of gene flow, selection is often strong enough to maintain population divergence and adaptation. Although topography and other environmental factors create dispersal barriers to gene flow patterns in plants, an important determinant of gene flow is the plant’s breeding system. Using many examples from California grasslands, Jain (1975) summarized some of the potential effects of plant breeding system on population structure. In general he found that reduced gene flow in inbreeding species results in greater among-population genetic divergence and lower levels of genetic variation within-populations (e.g., fewer polymorphic loci and reduced heterozygosity). He also suggested that reduced gene flow in selfing species facilitates local adaptation because selection does not have to be as strong to form ecotypes if gene flow is low.

The breeding system of a population or species can have manifold effects on local neighborhood structure and the likelihood that a mosaic of sub-populations or demes will develop (Levin and Kerster 1971). In a study on the inbreeding annual grass *A. barbata*, Rai and Jain (1982) examined how restricted gene flow creates patchy local genetic structure. They found that highly restricted gene flow created very small genetic neighborhoods (Wright 1943) that reduced effective population size. This reduction in
population size by restricted gene flow creates an environment where founder effects and genetic drift can be important in creating local genetic structure. This study suggests that much of the mosaic of genetic variation in molecular markers may represent random processes such as drift and not highly localized selection. Knapp and Rice (1996) found that regional and local allozyme variation in the less selfing native perennial grass *Elymus glaucus* (blue wildrye) conformed to expectations for a species with a more mixed mating system. Although *E. glaucus* is more self-pollinating than outcrossing (Cronquist et al 1977), Knapp and Rice (1996) still found substantial variation within populations with an average of 31.4% of loci polymorphic and an average of 1.4 alleles per locus. However, other aspects of population structure reflected the reduced gene flow associated with inbreeding populations. For example, gene flow was relatively low (Nm = 0.205) and most of the genetic variation was distributed among populations (54.9%).

In contrast, a survey by Hamrick and Godt (1989) found that genetic variation in outcrossing plant species occurred primarily within populations and averaged around 85.2%. Dyer and Rice (1997) studied the potential importance of differential dispersal between pollen and seeds in creating genetic structure in the native bunchgrass *Nassella pulchra* (purple needlegrass). Using phenological data from a common garden and molecular markers (i.e. RAPDs) in field populations, they examined how genetic differences scaled along highly localized gradients in soil depth up to the landscape level. They argued that local selection among soil microenvironments is facilitated by highly restricted gene flow resulting from limited seed dispersal distances. In contrast, weak genetic structure at the landscape level reflected the much greater dispersal distance of pollen: they argue that the detection of genetic structure is highly dependent on the spatial
scale of analysis. Both *E. glaucus* and *N. pulchra* are important species for restoration in California grasslands; these two studies suggest that patterns of gene flow in mixed mating systems complicate the determination of scales of adaptation.

Genetic variability within mixed mating systems does not appear to be related to gene flow. In studies of *Lupinus* (lupine), Harding and others (1974) found that genetic variability within populations did not determine outcrossing rates, although outcrossing rates were correlated with the degree of self-compatibility within each population. In studies of genetic variation in *A. barbata*, Rai and Jain (1982) predicted degrees of patchiness in known genotypes (demonstrated by variation in phenotype) based on measured seed flow and outcrossing rates among populations. The variety they found among the populations was significantly more than indicated by the predictions based on measured gene flow.

The ability of populations to accept pollen moved from other populations varies among many California native forb species, e.g., *Holocarpha macradenia* (Santa Cruz tarplant, Palmer 1982), *Eschscholzia californica* (California poppy, Cook 1961), and *Nemophila menziesii* (baby blue-eyes, Barr 2004). In experiments performed by these researchers, plants from some populations of these species exhibited potential outbreeding depression with reduced or no seed set when fertilized with pollen from other populations. Geologic processes may have influenced gene flow, enabling plants in regions of large land mass movement, such as the southern north coast ranges, to retain their ability to exchange genes across populations (Barr 2004). Nagy’s (1997) field experiment tested whether plants receiving foreign pollen (coastal x inland, inland x coastal) were able to produce seed. In this case, only the coastal subspecies *Gilia*
capitata subspecies chamissonis (dune gilia) was able to produce seed from ovules fertilized with foreign pollen, while inland populations of Gilia capitata subspecies capitata (no common name) were not able to hybridize when the other subspecies was the pollen donor. These results were repeated in a greenhouse study. In experiments where native pollen was diluted by foreign pollen, dilution resulted in a smaller seed set for both subspecies.

Simple geographic distance is an obvious barrier to gene flow among populations. In a Washington state study of molecular variation in the California vernal pool native Navarretia leucocephala (no common name), Boose et al. (2005) found that, generally, populations differed more strongly from one another the further apart they were. Pools a few hundred meters apart were not differentiated from one another for the RAPD molecular markers used, and estimates of number of migrants among populations were surprisingly high (1 to 4 migrants per year), given the island-like nature of the habitats in which these plants are found. Molecular differentiation was apparent when distances between pool populations became greater than about 1500m,. Interfertility also varied by geographic distance in Streptanthus glandulosus (jewelflower, Kruckeberg 1957), resulting in a revision of the genus (Kruckeberg 1958). Mayer et al. (1994) found high degrees of population differentiation using enzyme analysis, even within the most widespread species of Streptanthus. Estimates of the number of migrants between populations led to the conclusion that most of these serpentine endemics were vulnerable to genetic drift due to lack of gene flow. These researchers found a classic "isolation by distance" pattern within the Streptanthus species tested in these experiments. Morphology and interfertility patterns closely tracked the differentiation found in the
enzyme analysis. In studies of *Limnanthes* (meadowfoam) species, the allozyme-measured genetic distance between populations closely tracked their geographic distance (Jain 1981).

Genetic diversity within a population can interact with inbreeding depression to make a population more or less likely to interbreed with other populations. In a common garden experiment in Riverside, populations of different numbers of individuals (2, 5, 10, and 20) of *Raphanus sativus* (wild radish) in three levels of relatedness (full-sib, half-sib, and unrelated) were established (Goodell et al. 1997). A paternity exclusion analysis using allozymes determined the degree of outcrossing that occurred among the populations. Populations greater in size than two were indistinguishable from one another in the amount of out-sired seed they produced. The degree to which relatedness affected the proportion of out-sired seed differed among the three experimental trials and differed among the population sizes, but in population size 2, half-sib populations always had the greatest or not-significantly-different-from-greatest proportion of out-sired seed.

Gene flow events are easier to detect in small populations, and also easier to detect in genetically uniform populations. Because *R. sativus* has a self-incompatibility system with at least 32 alleles, genetically uniform populations were expected to have the highest out-siring rates. However, because paternity analysis was used, genetically uniform populations were also the most likely to exhibit out-siring rates. (An out-siring event in a genetically diverse population will not be detected if the out-population pollen donor has a genotype found in the home population.) In addition, distance between populations was small (<100m), and so the inability of small populations to effectively attract pollinators
was probably a muted effect. Nonetheless, this experiment showed that demography and relatedness can affect gene flow among populations and do so differently among years.

**The genetics of inbreeding**

In trying to explain the diversity of breeding systems in plants, there has been much speculation as to the impact of breeding system on the genetic structure of plant populations. In particular there has long been an interest in how breeding system may influence the capacity of plant populations to respond to natural selection. Plant evolutionary biologists have argued that inbreeding, by reducing both gene flow among populations as well as genetic variation within populations, should facilitate close adaptation to current selective regimes (Stebbins 1957). It was reasoned that lower gene flow among selfing populations would be less likely to swamp adaptive differentiation driven by selection. However, it was also assumed that the concomitant reduction in within-population variation would reduce the evolutionary potential of a population to respond to new selective challenges and might result in a populations or even species becoming evolutionary “dead ends”.

Research on selfing annuals in the California grassland was pivotal in testing theoretical predictions that inbreeding should increase among-population variation while reducing within-population genetic diversity. (Allard et al. 1968). Early work by Knowles (1943) on populations of *Bromus hordeaceus* (soft chess) across a climatic gradient from coastal to more interior sites in California supported some of the predictions but countered others. Knowles found clinal variation in quantitative traits such as flowering time, plant height, and tillering capacity as well as sharp differences
among populations along the cline. These distinct differences among populations were in accord with theoretical predictions but populations of *B. hordeaceus* also exhibited substantial within-population variation. Strong differences among families, indicative of within-population genetic variation, were found for all quantitative traits measured. In stark contrast to the theoretical predictions of genetic uniformity within populations of inbreeders, Knowles concluded from his study that most, if not all, *B. hordeaceus* individuals within each population were genetically distinct. Subsequent work on *B. hordeaceus* by Jain et al. (1970) also found substantial variation between and within populations for quantitative traits although levels of within-population variation differed sharply among populations. Interestingly, Jain et al. (1970) did not detect a clinal gradient in variation and they speculated that the day length regime of their common garden may have masked phenotypic expression of clinal variation.

The unexpectedly high rates of within–population variation found in both of these studies on *B. hordeaceus* was corroborated by work in *A. fatua* where similar differences among families were detected for morphological, phenological and fitness traits (Imam and Allard 1965). In *A. fatua*, statistical tests of family differences indicated that when families were compared for multiple traits, essentially every family differed from every other family, indicative of substantial genetic variation within populations. In addition to these differences among families, Imam and Allard (1965) also detected significant variation among individuals within full sib families that suggested that there may be heterozygosity within the parent individuals. To test for this possibility, they established high and low selection regimes on flowering time, height, and growth habit (e.g., prostrate vs. erect). After only a single generation of selection, they found significant
differences between low and high selection lines for all characters. They interpreted this rapid response to selection as strong evidence that individuals within inbreeding populations may still be heterozygous for quantitative traits. They concluded that phenotypic differences among full sibs within families of selfing species are not just caused by environmental or developmental factors but also by segregation of distinct genotypes.

A series of studies by Allard and his co-workers on the highly selfing native annual grass *Vulpia microstachys* (small fescue) further challenged the assumption that highly inbred species should exhibit little genetic variation within populations (Kannenberg and Allard 1967, Allard and Kannenberg 1968, Adams and Allard 1982). Focusing on *V. microstachys* as a model system for studying the effects of extreme inbreeding on genetic structure, Kannenberg and Allard (1967) initiated study of this native annual grass by collecting from populations located in three climatic regions in California (Inner Coast Range, Eastern edge of the Central Valley, Sierra Foothills). Using extensive progeny testing from this field collection, they examined mating system variation among populations as well as genetic structure. In general *V. microstachys* populations were found to exhibit extremely low rates of outcrossing (usually much less than 1%) with no detectable hybrids in the examination of over 20,000 individuals. Despite this extremely high level of selfing, a hierarchical analysis of phenotypic variation indicated that the greatest variation in traits such as flowering time, height and tillering rate was at the among family level; a result suggesting significant genetic variation within populations despite almost complete inbreeding. The authors also noted that expression of potential outcrossing (i.e. frequency of plants with extruded anthers)
varied significantly among populations and may be related to site quality such that outcrossing rates increase in favorable sites. Significant variation among individuals was also detected in some families but it was not possible to determine whether this variation reflected genetic segregation rather than variation in developmental homeostasis among homozygous families. The strong differences among families within a population occupying a spatially heterogeneous environment prompted Kannenberg and Allard (1967) to argue that this type of localized genetic structure may allow *V. microstachys* to adapt to selection varying over very small spatial scales.

Using electrophoretic markers, Adams and Allard (1982) confirmed the highly selfing nature of *V. microstachys*. However, they also detected what they called “bursts of outcrossing” in one of the populations that increased the frequency of heterozygotes from less than 1% to more than 9% in a single generation. Kannenberg and Allard (1967) had previously noted that because most individuals are homozygous for different alleles at many of their loci, any outcrossing event tends to produce a unique multiple heterozygote. Within-population diversity of homozygotes, when coupled with these sporadic episodes of outcrossing, could generate a significant amount of genetic diversity that could help to explain the high amounts of variation seen in this highly selfing annual.

Repeated assertions that fine-scale spatial genetic variation in *V. microstachys* represents the action of highly localized selection was not tested until a reciprocal transplant study at the U.C. McLaughlin Reserve in the Inner Coast Ranges of northern California (Jurjavcic et al. 2002). These authors were interested in understanding how gradients in stress (i.e. a serpentine soil gradient) and competition interact to determine *V. microstachys* distribution and abundance. They manipulated competition in three habitats
(rocky serpentine, serpentine meadows, nonserpentine grasslands) and also reciprocally transplanted seed sources among all 3 habitats. If *V. microstachys* distribution is dependent on fine-tuned genetic differentiation, then each habitat seed source should perform best in its “home site”. Contrary to the expectations of previous researchers, local adaptation was not strongly expressed in the study. Local adaptation was suggested by a higher emergence and survival of rocky serpentine genotypes in their home sites, but growth and survival did not show any seed source effects. Instead, variation in these fitness parameters across habitats was the result of phenotypic plasticity and not genetic differentiation. It thus appears that although *V. microstachys* may represent a model system for studying inbreeding, it is not an exemplar for demonstrating how restricted gene flow in selfers may facilitate highly localized adaptation.

**Variable outcrossing – causes and consequences**

Many outcrossing species are capable of self-fertilization, and the degree to which a species exhibits outcrossing may be variable. The ratio of outcrossing to inbreeding within a population informs both the genetic variability of the population and its ability to exchange genes with neighboring populations. In three populations located in the Sierran foothills (2 populations) and near Cache Creek Canyon, (County) Weil and Allard (1964) used two phenotypic characters under single gene control to examine outcrossing rates in *Collinsia heterophylla* (Chinese houses). By choosing two sites within each population to conduct their experiments, they were able to examine the degree of variability in outcrossing both within and between populations. While other (multigenic) phenotypic traits showed large differences in genetic variability within populations and between
sites, the differences were not attributable to variable outcrossing rates among populations and sites.

In a common garden experiment performed on seed collected from a variety of locations near Lake Berryessa, Horovitz and Harding (1972) studied the degree of outcrossing indirectly in *Lupinus nanus* subsp. *nanus* (no common name), recording floral characteristics that would make each plant more or less able to attract flying insects that would serve as cross-pollination vectors. As measured in an *ex situ* experiment, outcrossing rates varied considerably among individual plants (from 0 to 0.844), but less so on a population level (0.467 to 0.828). Outcrossing rates also varied over the season within phenotypes, generally increasing over the 60 day experimental period. While only maternal outcrossing rates were observed in this study, male outcrossing rates would also certainly be affected in either parallel or opposing manners. In natural populations of *L. nanus* subsup. *nanus* as well as the subspecies *menkerae, apricus, latifolium* and *vallicola*, outcrossing rates were still variable in three populations of subsp. *nanus*, ranging from 0.5 ± 0.2 to 0.83 ± 0.05, Harding et al. 1974). All subspecies exhibited variation among populations for outcrossing rates, some by a factor of 2 and others by a factor of 10.

A survey of 29 ruderal populations of *Lupinus succulentus* (arroyo lupine) in the coast ranges from Northern to Southern California, the Transverse ranges, and inland to the Sacramento Valley (Harding and Barnes 1977) showed heterozygosity rates ranging from 0.009 to 0.548 and outcrossing frequencies ranging from 0.138 to 0.971. The study also found year effects in outcrossing rates. There was nonetheless a strong correlation between outcrossing rates and genetic diversity within populations. In contrast, Harding
et al. (1974) found no correlation between outcrossing rates and genetic diversity within populations of *Lupinus*. Differences in ploidy level within and among populations of the same species may also influence outcrossing rates. In studies of *Delphinium gypsophilum* (gypsum-loving larkspur, Koontz and Soltis 2001), entire populations were found fixed for one ploidy or another, but some populations contained mixtures of both ploidy levels found within the species, which would significantly affect outbreeding rates between and within populations.

**Population Differentiation**

**Local molecular variation**

Gene flow among populations is often measured by examining molecular markers, however there has been little demonstrated correlation between adaptation and molecular genetic markers in California grassland species. It is important to note that molecular markers portray a long history of gene flow, and a recent interruption of historical gene flow may not be apparent from an analysis of molecular variation within populations. The most rigorous method of measuring current gene flow is by conducting paternal exclusion analysis on molecular markers within populations, but this technique has flaws: it underestimates outsiring events (Goodell et al. 1997), ignores year-to-year variation among annual plants, and is labor-intensive. While molecular differences among populations are often used to inform decisions on the conservation value of these populations, it is important to remember that molecular markers do not always diverge in parallel with phenotypic or adaptive characteristics which are ultimately the mechanism
for sustaining plant populations. For example, Linhart (1988) found adaptive differences among vernal pool populations of *Veronica peregrina* (purselane speedwell), this differentiation was not reflected in electrophoretic assays of enzyme systems (Keeler 1977). Instead differences between center and edge in terms of absolute polymorphism was apparent, with edge individuals exhibiting more electrophoretic variation than center individuals. This paralleled the morphological tests of Linhart (1988), who found more variation among individuals inhabiting edge habitats. Knapp and Rice (1998) found significant differentiation among populations of *N. pulchra* (purple needlegrass) in both morphological and isozyme traits when plants were grown in a common garden. However, in cluster analysis, populations grouped differently depending on which measure was used, indicating a lack of parallel between phenotypic traits and molecular markers.

There are some examples where variation in genetic markers is correlated with adaptive variation. In a reciprocal transplant experiment of known genotypes of *A. barbata*, Jain and Rai (1980) found a small but significant advantage of home genotypes vs. non-local genotypes. This example of genotypic variation paralleling local adaptation is also suggested in the myriad work of Allard (see above discussion of co-adapted gene complexes). One example is an examination of 14-locus genotypes (Allard 1996) found in *A. barbata* across two very different regions of Spain and across California. The presence of reorganization of common alleles in Spain into new genotypes in California may indicate a link between these complex genotypes and adaptation to growing conditions in California.
Molecular markers can be used to detect differences in heterozygosity among populations: in the case of *Trifolium amoenum* (showy Indian clover), an inland population (Occidental, Sonoma County) known to have been founded by a single individual was compared to a coastal population (Dillon Beach, Marin County) of this rare plant, and was also compared to eight populations of two congeneric species (Knapp and Connors 1999). As expected, the inland population showed reduced heterozygosity compared to the coastal population, while the within-population heterozygosity of the coastal population compared favorably to that of one of the comparison congeneric species. (The heterozygosity of the other comparison species was so low as to have been determined to have a significantly different mating system: probably primarily inbreeding in contrast to the other two outcrossing species.)

**Local adaptation**

Differences among populations are somewhat simple to test for: collect seeds from several populations, grow them in a common garden, and observe whether the resulting plants differ from one another on a population level. Without growing the populations together at a single site, it is impossible to tell whether morphological differences are due to a plastic response to the environment or due to a heritable factor, such as genetic differences or a maternal effect. Demonstrating that genetic differences among populations are adaptive is somewhat arduous in that seeds from a population must do better in their home environment than seeds from populations in other environments. Many studies have shown population differentiation without testing the adaptive nature of the differentiated traits. While differences among populations in seed
dormancy are common, examples including *Eschscholzia californica* (California poppy, Montalvo et al. 2002), *Nemophila menziesii* (baby blue-eyes, Cruden 1974), and *Blepharizonia plumosa* (big tarweed, Gregory et al. 2000), the genetic link of dormancy cues to environmental differences between the populations have not been shown. Population differences in seed dormancy and leaf pigmentation in *Eschscholzia californica* (California poppy, Espeland and Myatt 2001), and allozyme heterozygosity in *Trifolium amoenum* (showy Indian clover, Knapp and Connors 1999), and isozyme diversity within species of the *Streptanthus* (jewelweed) complex (Mayer et al. 1994) might be adaptive, but also may be a result of a genetic drift. Furthermore, small, isolated populations are less likely to have genetic and thus phenotypic diversity and are more likely to be differentiated from other populations in a nonadaptive manner.

**Coadapted gene complexes**

An important question in population genetics revolves around the relative importance of selection at the single gene level versus its potential effect on groups of genes or even whole blocks of chromosomes. Franklin and Lewontin (1970) argued that reduced rates of recombination can cause correlations among allelic states such that selection acts on correlated or interacting sets of loci (i.e. epistatic selection). Theoretical studies on inbreeding (Jain and Allard 1966) indicated that close inbreeding strongly reduced recombination rates among all loci (even unlinked loci) and resulted in dynamics similar to those of very tight linkage among genes. An early experimental examination of the effects of inbreeding on the evolution of allelic correlations was conducted by Clegg et al. (1972) on two populations of *Hordeum vulgare* (wild barley). They found that
significant departures from random association among loci increased over generations in both populations and that a similar multilocus gametic type was in excess in both populations by the 26th generation. The authors argued that these two results indicated a strong role for selection in the development of highly interactive, coadapted gene complexes; a concept first introduced and developed by Dobzhansky (1970).

In an extension of the work by Clegg and Allard (1972) on adaptation of multilocus genotypes of *A. barbata* to regional climate, Hamrick and Allard (1972) reported on the microgeographic variation of multilocus genotypes across a local moisture gradient at a site near Calistoga, (Napa County) California. They reported that the gradient appeared to be a mosaic of microsites differing in moisture availability and they argued that specific sets of alleles were correlated with these drier and wetter microsites (the “xeric” and “mesic” genotypes, respectively). The fact that alternate sets of alleles were associated with each genotype suggested the possibility that selection for co-adapted gene complexes may be occurring at a very local scale in this microhabitat mosaic. Citing the apparent association of multi-locus “xeric” and “mesic” genotypes with localized variation in soil moisture, Allard et al. (1972) proposed that these genotypes represent the product of selection for co-adapted gene complexes. They argued that a gametic-phase disequilibrium exists in these populations and that the co-adapted gene complexes of the “mesic” and “xeric” genotypes are maintained by both selection and reduced recombination caused by inbreeding and/or linkage.

From an ecological perspective, all of these studies were problematic. The determination of mesic and xeric microhabitats was not supported by independent and quantitative assessments of variation in soil moisture while mesic and xeric microsites
were determined by casual observations and phenotypic variation in plant vigor and appearance. In addition, the use of observations of phenotypic variation in *A. barbata* growth and vigor in the field to confirm mesic and xeric genotypes confounded genetic and environmental determinants of phenotypic expression. For example, field observations indicated that putative mesic genotypes flowered earlier, were taller, produced more tillers, and had higher seed production than xeric genotypes but these differences could also be caused by environmental variation in site conditions. In an effort to demonstrate that this phenotypic variation in the field had some genetic basis, Hamrick and Allard (1974) conducted a common garden experiment. Using two populations of each genotype, they found that mesic and xeric genotypes grown in a common garden were significantly different in flowering time, height, and tiller number but not in seed production. Assuming that maternal environmental effects were not significant, this study confirms that, except for seed production, the suite of phenotypic characters used to characterize mesic and xeric genotypes did have a genetic basis. Unfortunately, however, the approach used by Hamrick and Allard (1974) to select mesic and xeric genotypes from the field was again based on phenotypic variation in *A. barbata*. To relate the genetic variation found in the common garden to ecotypic adaptation to soil moisture, the authors should have first independently determined variation in soil moisture (e.g. gravimetric water content or soil moisture potential) and then collected and categorized plant material as to xeric or mesic on the basis of the soil moisture data. In an expansion of the original study, Hamrick and Holden (1979) examined a much larger number of sites and attempted to characterize in some detail the mosaic of putative mesic and xeric microsites. Although the authors suggested that some
soil data were taken, the quantitative classification of xeric and mesic microsites was again solely determined by phenotypic variation in *A. barbata* height and flowering panicle density. The use of phenotypic expression in the field to designate what is a xeric or mesic microsite again assumes that genotypes are closely tracking soil moisture variation; independent assessment of soil moisture variation is necessary to test this assumption. Although the authors argued that selection has shaped genetic structure within the soil moisture mosaic, they diverged from the conclusions of Allard et al. (1972) and admitted that there was insufficient evidence to confirm that epistatic selection was occurring and maintaining coadapted gene complexes in the xeric and mesic genotypes.

Clearly, a good test of the reality of fine-scaled adaptation to soil moisture variation would be provided by a field experiment where mesic and xeric genotypes are reciprocally transplanted between putative mesic and xeric microsites. Although this conclusive type of experiment has never been done on the microsite level in *A. barbata*, Jain and Rai (1980) used a reciprocal transplant approach to test local adaptation to regional climate. In a study that was rather remarkable in its scope and duration, they found some evidence for local adaptation at a regional level but the results indicated weak selection. In a long term experiment, many of the non-local colonies that they established persisted quite well throughout the course of the study (5-8 years depending on site) and the authors noted that there was often very little shift in genotypic frequency over time. A shorter term study of two common gardens established in a coastal and interior valley site also did not detect strong regional adaptation. However, there was an indication of weak selection for the mesic genotype in the cooler coastal site and
selection for the xeric genotype in the warmer interior site. Overall, the study indicated some regional adaptation, but selection was not as strong as expected. The results beg the question of whether selection is strong enough to create fine-tuned adaptation to the soil moisture mosaic at the Calistoga site in spite of gene flow among the microsites.

Reciprocal transplant experiments at the small spatial scale of the Calistoga site have not been done, but Gardner and Latta (2006) recently compared the relative fitness of mesic and xeric genotypes in a reciprocal transplant experiment across a regional rainfall gradient in northern California. Using the UC Hopland Research and Extension Center (Mendocino County) as a “mesic” planting site and the UC Sierra Field and Extension Center (Yuba County) as a “xeric” planting site, they found that the mesic genotype was more fit in both mesic and xeric sites. Although these relative fitness results are not strong evidence for local adaptation, previous work by Latta et al. (2004) have identified a suite of characters in the mesic and xeric genotypes that would suggest adaptation in A. barbata to variation in microsite productivity and soil moisture. They found that mesic genotypes were more competitive than xeric genotypes and, primarily because of greater seed size, seedling size and adult fecundity were also greater in the mesic genotype. On the other hand, the xeric genotype exhibited greater adaptation to soil moisture stress by expressing a greater root mass ratio and allocating significantly more root biomass deeper in the soil.

**Phenotypic plasticity and adaptation to heterogeneous environments**

A hallmark of the California grassland ecosystem is its wide variation in weather patterns from one year to the next and pronounced spatial heterogeneity at multiple
scales. Two alternate strategies that may allow plants to adapt to such rampant environmental heterogeneity are selective genetic differentiation (i.e. “classic” local adaptation) or an environmentally induced phenotypic response (i.e. adaptive phenotypic plasticity). In systems with fine-grained heterogeneity where temporal variation is rapid relative to an individual’s generation time, theory predicts that adaptive plasticity should be the primary adaptive response to a variable selective regime (Levins 1968). The potential importance of phenotypic plasticity in plants as an alternate strategy to genetic differentiation in response to selection was first summarized by Bradshaw (1965). Subsequent reviews by Schlichting (1986) and Sultan (1987) have further emphasized the importance of phenotypic plasticity as a major force in plant evolution by highlighting additional concepts such as correlated trait matrices and the capacity of selective buffering by plasticity to maintain genetic variation.

Researchers were quick to appreciate the potential importance of phenotypic plasticity as an adaptive strategy in California grasslands, especially in introduced species that expressed low amounts of heritable variation because of genetic bottlenecks and inbreeding. Marshall and Jain (1968) compared phenotypic plasticity between *Avena fatua* and *A. barbata* in two greenhouse studies that examined phenotypic responses of both grasses to variation in soil characteristics and levels of intraspecific and interspecific competition. Because *A. fatua* had been shown to be more genetically variable than *A. barbata*, they hypothesized that the genetically less variable *A. barbata* should exhibit greater plasticity. This prediction of an apparent trade-off between genetic differentiation and plasticity was supported by results indicating that the genetically depauperate *A. barbata* was more plastic across environments for essentially all traits measured.
Compared to the typical agricultural habitat of *A. fatua*, Marshall and Jain (1968) further suggested that the grassland environment of *A. barbata* may be more unpredictable and thus selects for greater plasticity. The importance of plasticity in generating regional patterns of phenotypic variation was studied in the highly selfing annual grass *Bromus rubens* (red brome) by comparing variation in field populations with progeny raised in a controlled environment (Wu and Jain 1978). They found that only a small amount of the regional phenotypic variation in traits ranging from flowering date to seed weight could be attributed to genetic variation (12-27%) while the bulk of variation (73-88%) represented plastic responses. Similar to *A. fatua* and *A. barbata*, a comparison of *B. rubens* and *B. hordeaceus* suggested that plasticity is greater in the more genetically depauperate *B. rubens* while regional adaptation in the more genetically variable *B. hordeaceus* results from local genetic differentiation (Jain 1979). These congeneric comparisons support the concept of alternate adaptive strategies and suggest a cost to plasticity (e.g., “jack of all trades, master of none”). However, although a cost to plasticity is often assumed, it is rarely demonstrated and only recently have approaches been developed that may be able to get at this very difficult question (De Witt et al. 1998).

In a series of field experiments, Platenkamp (1990, 1991) examined the importance of phenotypic plasticity in the demography of an introduced perennial grass species *Anthoxanthum odoratum* in two sites that differed in soil moisture availability. Using reciprocal transplants of both seeds and clonal replicates, he examined the relative importance of genetically based local adaptation and adaptive plasticity in structuring mesic and xeric populations in a coastal prairie. In an examination of seed and seedling
traits (Platenkamp 1991), variation in germination seemed to exhibit some genetic basis because seeds from the mesic site exhibited higher dormancy regardless of planting site. However, there was no indication of genetically based local adaptation. Overall, phenotypic plasticity was the primary determinant of differential seedling survival and eventual fecundity within mesic and xeric sites. In another experiment, Platenkamp (1990) reciprocally transplanted clonal replicates of *A. odoratum* between the mesic and xeric site to more precisely examine the relative importance of phenotypic plasticity and genotypic differentiation in local adaptation. Similar to results from the study on seed and seedling characteristics, there was no indication of local adaptation (i.e. no site by clone origin interactions). Although the relative importance of genetic differences and plasticity were somewhat trait dependent, fitness variation measured by mortality and cumulative fecundity over a three year period was almost entirely caused by phenotypic plasticity. Platenkamp (1990) suggests that the failure to demonstrate a clear pattern of local adaptation may reflect the recent introduction of this species to the site as well as year to year fluctuations in the soil moisture gradient. The importance of phenotypic plasticity in this species was further demonstrated by a study of the effect of neighbors on *A. odoratum* fitness (Platenkamp and Foin 1990). Although the density and biomass of competitive neighborhoods strongly influenced growth and cumulative reproductive output of clonal replicates, there was no difference among clones in response to neighbors. This lack of genetic variation in response to neighborhood composition indicates that response to competition in this species is entirely plastic.

Germination timing and seed dormancy are life history traits that can have far reaching effects on both year to year variation in community composition of California
grasslands (Heady 1958, Bartolome 1979) and population dynamics (Rice 1989). A study by Jain (1982) on regional variation in seed dormancy in several annual species suggested that for the grasses tested, there was a genetic adaptive response to the probability of significant summer rainfall. However, Jain (1982) also found that heritability for dormancy was low for some species and that phenotypic plasticity in germination was also important. A study by Rice (1985) on germination cueing in *Erodium brachycarpum* and *E. botrys* demonstrated that entirely plastic responses in seed germination had significant fitness consequences in terms of both survival and reproduction. He found that *Erodium* (storksbill or filaree) seeds germinated more readily under summer soil temperature regimes characterized by large diurnal fluctuations in temperature. This type of temperature environment is usually associated with reduced litter cover and grass density that, in turn, represents a favorable site for *Erodium* survival, growth and reproduction. This type of germination cueing represents an adaptively plastic response in germination behavior that has been documented in other habitats characterized by spatial or temporal variability in “windows of opportunity” for seedling recruitment (Venable and Brown 1988).

In addition to within-generation plastic responses in germination, there can also be “trans-generational” plasticity, where the environment of the maternal plant can influence the germination behavior of progeny. Although the potential adaptive nature of these maternal environmental effects is just beginning to be explored, it is clear that they can often exert significant effects on progeny fitness, especially at early stages of development (Mousseau and Fox 1998). An example from the California grassland is provided by a study on how maternal effects in *Nemophila menziesii* (baby blue-eyes)
can influence seed characters and, in turn, the fitness of the progeny (Platenkamp and Shaw 1993). They found that the maternal competitive regime had a strong influence on all the seed characteristics that they studied. Seed weight was reduced when the maternal plant was subjected to strong competition. In addition, seed dormancy and time to germination were increased in progeny from mothers that experienced competition. Delayed emergence time has been shown repeatedly to result in a competitive disadvantage to the seedlings that emerge later (Miller 1987, Rice and Dyer 2001, Ross and Harper 1972). Additionally, increases in seed dormancy can effectively lengthen the generation time of a population which, in turn, can reduce fitness by decreasing rates of population increase (Lewontin 1965, Rice 1989). The authors noted that the influence of the maternal competitive environment on all these seed traits represented a cross-generation extension of the negative effects of competition.

Finally, recent interest in the evolution of plasticity (Via et al 1995) has highlighted the fact that phenotypic plasticity like any trait can evolve if there is sufficient genetic variation for plasticity within the population. One of the earliest studies on genetic variation in plasticity in plants was conducted by Jain (1978) where he examined inheritance of phenotypic plasticity in *Bromus hordeaceus*. Using a parent-progeny regression approach, he found significant genetic control of plasticity for two populations that differed in genetic polymorphism (as determined by allozyme markers). Surprisingly rare, these types of studies that demonstrate the heritability of plasticity are critical for arguing the evolution of alternate strategies of adaptation to environmental heterogeneity.
Adaptation to Abiotic Gradients

California, with its great diversity in climate and soil types, provides a mosaic of conditions under which plants must thrive. As reviewed above, some species use adaptive phenotypic plasticity to perform well in a variety of environments, others depend on extremely localized adaptation to microenvironmental variability. Broader abiotic gradients have also been the subject of some research on ecotypic variation in California plants.

Serpentine soil gradients

Plants that grow on serpentine soil, in particular, have attracted much attention due to the harsh soil conditions (low in calcium and high in toxic metals) and high biodiversity on these soils. Serpentine soils can occur as islands of varying sizes within a matrix of more hospitable soils (see Harrison chapter in this book). As mentioned above, work by Jurjavcic et al. (2002) confirmed serpentine adaptation on rocky substrates in \textit{Vulpia microstachys}. Work by Kruckeberg (1951) demonstrated serpentine tolerance within races of \textit{Streptanthus} (jewelweed, subspecies \textit{glandulosus secundus} and \textit{glandulosus typicus}) in container experiments conducted outdoors on populations of seeds collected from serpentine and non-serpentine habitats. Soil type did not affect seed germination, but did affect growth in these species. In the same experiments, \textit{Gilia capitata} (no common name) segregated into serpentine tolerant and intolerant races even at the germination stage. In these experiments, \textit{Gilia capitata} and \textit{Achillea borealis} (yarrow, or, milfoil) were grown on both serpentine and nonserpentine substrates (the \textit{Streptanthus} was grown only on serpentine). Serpentine tolerant races of \textit{G. capitata} had
better germination and growth in non-serpentine soil than on their home soil. Similar results were achieved for *A. borealis*, although some nonserpentine strains showed partial tolerance to serpentine soils. These experiments tested plant-soil relationships to germination and growth and did not test adaptation *in situ* or in competitive environments. In a study comparing sandstone and serpentine ecotypes of *Bromus hordeaceus*, Frietas and Mooney (1996) found that serpentine ecotypes performed better under water stress, and both ecotypes performed poorly on sandstone soils when stressed for water.

Gradients can also occur within the serpentine soil type: *Lasthenia* (goldfields, an outcrossing genus) has evolved in a dichotomous manner (to mesic, ionically challenging soils, or to xeric, benign soils) on serpentine soils multiple times (Rajakaruna et al. 2003a). Within each subspecies tested (*L. californica* subsp. *californica* and *L. californica* subsp. *gracilis*, no common names) races with differing flavonoid compounds correlated with each soil moisture type were found. The flavonoid compounds did not correspond with phylogenies created with nuclear ribosomal ITS regions (Rajakaruna and Bohm 1999, Rajakaruna et al. 2003), indicating that the flavonoid compounds corresponding to soil type have arisen multiple times via adaptation to soil type. These two races (mesic and xeric) are found in close proximity at Jasper Ridge, and thus are an example of highly localized adaptation in an outcrossing species where low rates of selfing reduce the chance for the formation of coadapted gene complexes. It is interesting to note that although flavonoid compound signatures were specific to soil type, and each drought-adapted race did have higher fecundity in its own soil type than the mesic soil type (Rajakaruna et al. 2003b). No direct mechanistic explanation has been provided for
a relationship between flavonoid compound signature and adaptation to either drought stress or soil fertility.

**Coastal vs. inland climatic regimes**

The coast, with its moderate temperatures and summer moisture in the form of fog, is very different from the inland areas of California with hot, dry summers and colder winters, and this abiotic gradient has also been the subject of the study of ecotypic variation in California grassland species. Coastal and inland ecotypes have been found in *Gilia capitata*, *Bromus hordeaceus*, and *Eschscholzia californica* (California poppy) by performing reciprocal transplant experiments (Nagy and Rice 1997, Knowles 1943, Leger 2004, respectively). Coastal forms of *E. californica* tend to be prostrate and perennial with yellow flowers, whereas inland forms can be a mix of perennial and annual plants, with flower color ranging from yellow to orange. These differences between forms persist when grown in common garden environments (Leger 2004). It is likely that many factors contribute to the evolution of coastal and inland ecotypes of a species, as many abiotic factors differ between the coastal and inland environments in California. For example, water may be more available in the summer on the coast via fog compared to inland (Corbin et al. 2005). In addition, the coast is windier than inland, encouraging more prostrate growth forms. Biotic factors, such as pollinator availability, that are influenced by abiotic factors are also likely to come into play when assessing the adaptive nature of the coastal vs. inland ecotype.
Adaptation to Biotic Selection

Evolution of competitive interactions

As has been true for several other research areas in population genetics, the first studies of the evolution of competition were conducted on agronomic relatives of California grassland species. Allard and Adams (1969) used a composite cross of four barley varieties initiated by Suneson (1956) to examine changes in the frequency of each variety in response to intergenotypic competition. They found that after 18 generations of mass propagation, selection appeared to preserve genotypes that interacted in a synergistic fashion. They argued that this type of synergistic behavior is not predicted from genotype performance in pure stands and may be an important factor in maintaining genetic diversity within populations that contain mixtures of genotypes.

The role of genetic polymorphism in affecting the outcome of competition as well as the co-evolutionary history of competitors was examined for the introduced oat species *Avena fatua* and *A. barbata* (Yazdi-Samadi and Jain 1978). Collecting material from both mixed and pure stands of each species, they set up competition trials using populations with high and low levels of polymorphism. In general, higher levels of polymorphism favored either species when in competition with a monomorphic population of the other species; competition between monomorphic populations was not as severe. The authors noted that the experiments indicated a higher probability of coexistence between monomorphic populations and that this was consistent with field observations because most mixed stands of these species are composed of populations with low genetic variability. They also noted that there was low stability and more
unpredictable competitive outcomes in mixtures of populations collected from pure stands in the field. They suggested that this greater competitive indeterminism in allopatric populations may reflect a lack of co-adaptedness and co-evolutionary history between competitors.

Further evidence for the potential importance of co-evolution of competitors was provided by Martin and Harding (1981) in a study on competition between allopatric and sympatric populations of *Erodium cicutarium* and *E. brachycarpum* (storksbill or filaree). In addition to competitive mixtures containing allopatric and sympatric populations of each species, they also examined mixtures of transposed populations. Transposed mixtures were created by mixing populations from sympatric sites. For example, in their collection of *Erodium* from various field sites, Martin and Harding (1981) collected material from two sympatric sites, Tehachapi and Turtle Rock. To create transposed mixtures they planted *E. cicutarium* from Tehachapi with *E. brachycarpum* from Turtle Rock and vice versa. Results strongly supported the importance of co-evolutionary history in predicting outcomes of interspecific competition in *Erodium*: reproductive rates of sympatric mixtures were consistently higher than mixtures of allopatric populations and suggested evolution of resource partitioning. Further, reproductive output from transposed mixtures indicated that two of the three tested populations of *E. brachycarpum* responded specifically to the gene pool of its sympatric interspecific competitor.

As a counter-point to these studies indicating substantial selective response to competition, it should be noted that Platenkamp and Foin (1990) found no selective response in *Anthoxanthum odoratum* to variation in its competitive environment. In a
series of neighborhood manipulations, they examined response of *A. odoratum* genotypes to intraspecific competition (allographic and sympatric populations) as well as interspecific competition from *Holcus lanatus*. Reproductive output of *A. odoratum* decreased with competition but this reduction in reproduction did not differ among plants from different competitive neighborhoods; the increase in fitness appeared to be entirely caused by phenotypic plasticity.

**Other biotic interactions as agents of selection**

Direct interactions between plants certainly drive evolution, but indirect competitive interactions such as differential response to disease (Lawrence 1945) and to local pollinator activity (Horovitz and Harding 1972), may also serve to differentiate populations. Tests on *Deschampsia caespitosa* (Lawrence 1945) showed that resistance to rust varied by the seed source population, and also by the location in which the plants were grown: plants collected from Sierran meadows died from rust when grown at Stanford University, but all California collections were rust resistant when grown at Mather field and at a high-elevation location. In the case of *Lupinus* (lupine, Horovitz and Harding 1972), differences among populations were found in blue reflectance, honey guide patterns, flower length, and number of pollen grains per flower. Differences in outcrossing rates among these populations were positively correlated with low pollen production, but flower size and honey guides were unrelated to the degree of outcrossing found within populations.

Soil microbial communities are another biotic factor that may exert selective pressure on populations. Although this biotic factor on tree population dynamics is
beginning to receive some scientific attention, the research investigating soil microbial community as a selective influence on California’s grassland plants has yet to be conducted (but see Batten 2004).

Conclusions

Many of the important contributions to basic theory of plant population genetics were developed in California, using species collected within the state. Important concepts such as coadapted gene complexes, and speed and spatial scale of adaptation, were examined. Given the agronomic background of the scientists studying these concepts, this seminal work was primarily performed using convenient exotic species (primarily annuals). These exotic species were mostly invasive, and the research has contributed much to global scientific knowledge about basic population genetics of plant invasions. Most population genetic studies in California grasslands have not focused on native species. Thus, genetic aspects of persistence in a fragmenting habitat and species cohesion within a dynamic landscape have not been examined. Studies that have observed adaptation have generally focused on abiotic selective factors, and there is a paucity of work on how ecology (particularly interspecific interactions) may drive evolution in California grasslands. Nevertheless, of all the North American systems under study, California’s grasslands have probably had the most (but still sparse) attention paid to their competitive interactions and how they may drive evolution. We still have little understanding of important genetic processes for native species used for restoration. We know that populations within a species can be genetically different from one another in ways that might be important. We need to know how population genetics
affect the persistence of restored populations. Restoration projects successful over the long term are few and typically use only a handful of individual species. We also need to know how population genetics affects the persistence of native populations within the California grasslands, as many of these populations are prone to decline through land conversion and further habitat fragmentation.

Adaptation and restoration (BOX 1)

Recommendations for taking population genetics into account during restoration of native biodiversity. Adapted from McKay et al. 2005.

1) Collect locally if possible.
   
a. Seed collected from sites dissimilar (either in known or unknown ways) from the restoration site may be maladapted to the restoration site.
   
b. Populations close to the site will be less detrimentally affected by gene flow from the restoration site than if nonlocal seed is used.
   
c. Increasing the possibility of gene flow between the restoration site and local native populations will reduce the possibility of genetic drift and increase the ability of the restored population to respond to selection. (Populations often vary in their ability to interbreed, and populations further away from one another are more likely to be limited in their ability to interchange genes.)
2) Look for seed collection sites that match the restoration site in climatic and environmental conditions.

   a. Combine elevation and composite climate indices to establish environmental similarity.

   b. Information on climatic zones can be found in the Western Garden Book (Brenzel 2001), and a large “seed-zone” literature exists for tree species.

   c. Sampling across major ecological gradients may be another approach: preserving the allelic variation within the species at the restoration site (but see #1 and #3b).

3) Determine the breeding system(s) of the species under restoration.

   a. Highly selfing species are less likely to create problems of unwanted gene flow to neighboring populations, are less likely to suffer from inbreeding depression, but are also more likely to be locally differentiated.

   b. Highly outcrossing species are less likely to be locally differentiated, but are more likely to suffer from inbreeding depression and Allee effects. If seed is poorly selected, there may be problems with gene flow to neighboring native populations.

   c. Simple bagging experiments can show if a species is self-compatible; pollen-ovule ratios (Cruden 1977) can also give crude estimates of outcrossing rates.

4) Determine the ploidy level of the species.
a. Differences among populations for ploidy levels can occur, and can often prevent interbreeding. These differences can, in effect, turn a single restoration population into many small inbreeding populations.

b. Chromosome counts for species can be found in the Jepson manual (Hickman 1993), or a search of the cytogenetics literature may indicate if this is a cause for concern in the particular species under restoration.

5) Minimize “unconscious” selection when supplementing with commercial seed.

a. Select the commercial grower using the same criteria as one would use for other seed collection sites (see #2).

b. Harvest from the entire commercial population and over multiple years.

6) Make an effort to increase collective knowledge about the effects of population genetics on restoration success.

a. Track the performance (growth, survival, reproduction) of individuals of known seed sources within the restoration project.

b. Make this information available to other researchers and practitioners.

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