expression of dominant genes in duplicated sets of chromosomes (see Crow and Kimura, 1965; Otto and Marks, 1996).

Level of genetic load also appears to be related to the colonizing ability of individuals, with successful colonizers generally harboring little or no genetic load and non-colonizers harboring significant levels. See more details in Section 4.5.

Is there evidence of homologous chromosome pairing in sexually reproducing, diploid homosporous ferns? The hypothesis that at least occasional homologous chromosome pairing occurs to "release" genetic variation stored across duplicated chromosome sets (Klekowski, 1972a, 1979) was predicated on the hypothesis that all homosporous ferns are polyploids. The advent of isozyme electrophoresis and its application to ferns and lycophytes marked a major milestone in our understanding of many aspects of the nature of fern and lycophyte genetics. One of the most important findings was that species with the base chromosome number (x) for their group (i.e., genus and/or family) produced isozymic patterns that were consistent with them being genetic diploids (Gastony and Gottlieb, 1985; also see Haufler, 1987, 2002, and Haufler and Soltis, 1986 for thorough reviews and discussions). Species with greater than 2x sets of chromosomes typically exhibit isozymic expression that is consistent with genetic polyploidy. Among sexually reproducing ferns, evidence of homologous chromosomal pairing and recombination has only been discovered in a genetic polyploid, Ceratopteris thalictroides (Hickok, 1978a, 1978b). Otherwise, convincing evidence of homoeologous pairing has only been demonstrated for the apogamous triploid Dryopteris nipponensis (Ishikawa et al., 2003).

Do populations of homosporous ferns exhibit low levels of genetic diversity? The most commonly used technique for assessing population genetic diversity in ferns and lycophytes has been enzyme (also called isozyme or allozyme) electrophoresis. Commonly estimated measures of population genetic diversity are the mean number of alleles per locus (A), the percentage of loci examined that are polymorphic (P), and the expected heterozygosity assuming Hardy–Weinberg equilibrium (He). Table 4.1 shows data for 49 taxa of sexually reproducing, diploid, homosporous ferns for which values of these three parameters were available in the references cited or for which we could calculate values from the data given. Across all 49 taxa, mean A was 1.6 (range 1.0–2.8), mean P was 38.4 (range 0–80), and mean He was 0.182 (range 0.000–0.345). These mean values are similar to, and possibly higher than, those reported for populations of 468 species of seed plants (Table 4.1) reported by Hamrick and Godt (1990). Homosporous ferns as a group, therefore, do not harbor lower levels of genetic diversity than has been found in seed plants.
have significantly higher chromosome numbers compared to their close heterosporous relatives and seed plants.

Klekowski and Baker (1966) sought to explain the high chromosome numbers of homosporous ferns as a mechanism for maintaining heterozygosity in a putatively inbreeding group of plants. Because homosporous ferns are capable of producing hermaphroditic gametophytes, self-fertilization of these gametophytes (intragametophytic self-fertilization) results in completely homozygous sporophytes (see Chapter 2). Therefore, homosporous ferns potentially suffer severe losses of heterozygosity more frequently than do heterosporous plants. Based on these observations, Klekowski and Baker (1966) proposed that homosporous ferns acquired their high chromosome numbers in response to selection for increased heterozygosity via pairing of homologous chromosomes derived from polyploidization instead of conventional pairing of homologous chromosomes. According to Klekowski and Baker (1966), heterosporous ferns have lower chromosome numbers than homosporous ferns because they are obligately outcrossing and therefore cannot experience sharp reductions in heterozygosity through intragametophytic selfing. A variety of subsequent studies supported Klekowski’s hypothesis. For example, Hickok and Klekowski (1974) and Hickok (1978) demonstrated homologous pairing in a small percentage (<1–3%) of self-fertilized offspring of the tetraploid _Ceratopteris thalictroides_. These studies demonstrated that homologous pairing can occur and may operate to promote heterozygosity in homosporous ferns.

Isozymes were an important tool in evaluating Klekowski’s hypothesis and characterizing fern and lycophyte genomes. An early isozyme study allegedly showed support for the Klekowski and Baker hypothesis by demonstrating multiple bands inherited in non-Mendelian fashion (Chapman et al., 1979). However, a subsequent investigation demonstrated that a homosporous fern with the lowest (i.e., base) number for its genus (what is generally considered a diploid species) possessed a diploid, not polyploid, gene expression profile with Mendelian inheritance (Gastony and Gottlieb, 1982). Furthermore, it was demonstrated that the complex isozyme banding patterns of Chapman et al. (1979) were in part attributable to subcellularly compartmentalized isoymes (Gastony and Darrow, 1983; Wolf et al., 1987). Continued isozyme investigations supported the observation that homosporous ferns with base chromosome numbers for their genus have diploid expression profiles despite possessing relatively high chromosome numbers (Gastony and Gottlieb, 1985; Haufler and Soltis, 1986; Soltis, 1986; Haufler, 1987). These results differed from those in angiosperms, which showed that angiosperms with high chromosome numbers did indeed have duplicated sets of isozymes (reviewed in Gottlieb, 1982; Soltis and Soltis, 1990).
Analyses of natural fern populations also showed that they are predominantly outcrossing instead of engaging in intragametophytic selfing. Haufler and Soltis (1984) found that 83% of the examined populations of diploid *Bommeria hispida* had heterozygous enzyme banding patterns attributable to outcrossing between genetically different gametophytes. In a more extensive analysis of diploid populations of *Pellaea andremotifolia*, Gastony and Gottlieb (1985) determined that at least 81.3% of 145 sporophytes examined from natural populations arose through outcrossing between gametophytes carrying different alleles. Holsinger (1987) developed a statistical technique for estimating rates of self-fertilization in homosporous ferns and applied this to the data of Gastony and Gottlieb (1985). He found that there is no evidence of intragametophytic self-fertilization in the two populations with large enough sample sizes for his statistical analyses and concluded that intragametophytic self-fertilization occurs only a small fraction of the time, if at all. Thus Klekowski and Baker's (1966) original rationale for polyploid homologous heterozygosity, high levels of intragametophytic self-fertilization, was rejected.

To explain the paradox of high chromosome numbers and diploid isozyme expression in homosporous ferns, Haufler (1987) hypothesized that homosporous ferns may have acquired high chromosome numbers with diploid gene expression through repeated cycles of polyploidization and subsequent gene silencing without the loss of chromosomes. If such a mechanism is common, the genomes of extant homosporous ferns should contain multiple, silenced copies of each nuclear gene. In support of Haufler's hypothesis, a few studies have identified silenced nuclear genes in homosporous fern genomes. Gastony (1991) observed the process of gene silencing by documenting that the duplicated expression of a phosphoglucoisomerase isozyme in the neotetraploid homosporous fern *Pellaea rufa* progressively diminished to a diploid level in several populations. However, isozyme analyses can assess only the expression of enzyme-coding loci and not the presence of these genes. Therefore, this method cannot determine whether homosporous ferns with the lowest chromosome numbers in their genus have diploid expression of isozyme loci because they have never experienced polyploidization or because they have gone through the cycle of polyploidization and gene silencing, unless sequences of gene copies are examined (reviewed in Soltis and Soltis, 1987). Using DNA sequence based probes for chlorophyll a/b binding protein (CAB) genes in the homosporous fern *Polystichum munitum*, Pichersky et al. (1990) identified several copies of this high copy number gene and found that several gene copies have been pseudogenized, consistent with Haufler's hypothesis.

Subsequent studies evaluating gene copy number in homosporous ferns utilized restriction fragment length polymorphisms (RFLPs). RFLPs estimate the number of copies of a gene in a genome. This method has been used to analyze the genome of the homosporous fern *Pellaea patens*, and it was found that the gene for chlorophyll a/b binding protein (CAB) was duplicated in the genome, consistent with Haufler's hypothesis of polyploidization and subsequent gene silencing.
Figure 9.3 Kaplan-Meier survivorship curves of the gametophytes of five species over a 25 month study period. No data were collected during months 4-7 and 15-24. Open symbols indicate terrestrial species and closed symbols indicate epiphytic and hemi-epiphytic species. *Campyloneurum brevifolium* (Lodd. ex Link) Link and *Vittaria lineata* (L.) Sm. are epiphytes that commonly occur in the inner canopy and in high-light understory habitats. *Lomariopsis vestita* F. Myn. is an abundant hemi-epiphyte that occurs in the understory of both primary and secondary forests throughout the study site. *Danaea wendlandii* Reb. f. is a terrestrial species typical of more stable understory habitats of primary forests. *Pityrogramma tarta* (Cass.) Link is an abundant species often found in disturbed sites such as trail and road sides and on the root balls of recently fallen trees.

settings. Provided that fertilization and more importantly, disturbance, is limited, many terrestrial species can also live for years (Figure 9.3).

Increased longevity generally results in increased gametophyte size. Gametophytes of many square centimeters that can produce dozens of sporophytes in space and time are not an uncommon occurrence in tropical forests (Watkins, personal observations). Even in the case of many temperate species, gametophytes have been shown to over-winter (Pickett, 1914; Sakai, 1980; Farrar and Gooch, 1975; Sato and Sakai, 1981). Such observations call for fundamental revision of the traditional view of gametophyte longevity and sporophyte production. Although the view that gametophytes are short lived and produce a single sporophyte may be applicable to many temperate and tropical terrestrial species, this perspective should be reconsidered in the broader context of fern and lycophyte diversity and evolution.

The role of habitat disturbance provides another example of differences between epiphytic and terrestrial gametophyte species. Recent studies by Watkins et al. (2007a) have confirmed earlier studies (Peck, 1980; Peck et al., 1990) showing the general dependence of terrestrial gametophytes upon disturbance