Background of the Study

It is generally accepted that genetic variation is essential for evolutionary processes. Mutation, selection, gene flow and random drift are the main factors influencing the level and structure of genetic variation within and among natural populations, and, therefore, may have important evolutionary consequences. In addition to these factors also the genetic system of a species, such as the breeding system, has been shown to affect the genetic structure of natural plant populations (Hamrick & Godt, 1990). This thesis aimed at getting insight into the factors that determine the genetic structure of bryophyte species, because, due to the unique predominantly haploid life cycle and the (assumed) predominance of asexual reproduction in bryophytes, these factors might differ between bryophytes and vascular plant species (Longton, 1976; Mishler, 1988; During, 1990; Emmos, 1990). For this reason the level and structure of genetic variation has been investigated for five species of the moss genus Polytrichum (P. commune, P. formosum, P. piliferum, P. juniperinum and P. longisetum) at three hierarchical levels: among species, among populations within species and within populations. These studies were performed by using allozyme electrophoresis and microsatellite analysis as the main tools.

Genetic Variation among Species

Genetic variation among Polytrichum species has been studied because (i) phylogenetic relationships among the examined taxa might elucidate disputed taxonomic relationships within this genus, (ii) the level of genetic divergence between the studied Polytrichum species might be indicative for their evolutionary rate, and (iii) differences in the level of genetic variation between the examined species might reveal relationships between the level of genetic variation and both life-history characteristics as well as the genetic system.

Phylogeny

For several Polytrichum species, for example P. commune s.l. and P. juniperinum s.l., infraspecific taxa have been described. However, using morphological characters only, bryologists have not (yet) reached consensus on the taxonomic status of these taxa (Schriebel, 1982, 1991; Wyatt & Derda, 1997). For instance, P. commune var. commune and P. commune var. uliginosum have traditionally been recognised as varieties within the species P. commune s.l. Using allozyme electrophoresis, however, it is shown in the chapters 2 & 4 that Nei’s (1987) genetic identity (I = 0.61) between these two taxa is comparable to those generally observed for congeneric moss species (see Table 2.5 of chapter 2). Moreover, 6 of the 14 analysed enzyme loci showed alleles that were diagnostic for each of these two taxa, and no hybrid genotypes, i.e. genotypes showing diagnostic alleles of both taxa simultaneously, have ever been observed (chapter 2). An across-species analysis of microsatellites (chapter 3) that have been developed specifically for P. formosum (chapter 5) did also indicate that P. commune var. commune and P. commune var. uliginosum are genetically rather different. For these reasons, also taking into account the differences in morphology and habitat association, these two taxa should
be regarded as two distinct species: \textit{P. commune} and \textit{P. uliginosum} (chapter 2). In addition to this study, high levels of genetic divergence have also been observed between other pairs of bryophyte taxa, for example between \textit{P. commune} and \textit{P. jensenii} (Derda & Wyatt, 1999a), and between \textit{P. juniperinum} and \textit{P. strictum} (M. van der Velde, unpubl. data; Derda & Wyatt, unpubl. ms.), that previously have been regarded as conspecific taxa of \textit{P. commune s.l.} and \textit{P. juniperinum s.l.} (e.g. Crum & Anderson, 1981). This suggest that also to the taxa \textit{P. jensenii} and \textit{P. strictum} species rank could be assigned (Derda & Wyatt, 1999a, unpubl. ms.).

As several different classifications of the Polytrichaceae have been proposed (Smith, 1971; Koponen et al., 1977; Anderson et al., 1990), it is clear that for the taxonomy of this family also on the generic level no unanimity exists among bryologists. Nevertheless, many bryologists follow the classification of Smith (1971), who proposed the division of \textit{Polytrichum} s.l. into the genera \textit{Polytrichum s.s.} and \textit{Polytrichastrum}. As \textit{P. formosum} and \textit{P. longisetum} are tentative \textit{Polytrichastrum} species (Smith, 1971), the low levels of Nei’s (1987) genetic identity \((I = 0.13-0.24)\) observed between \textit{P. formosum} and each of the species \textit{P. commune}, \textit{P. uliginosum}, \textit{P. juniperinum} and \textit{P. piliferum} (chapters 2 & 4), and the close relationship between \textit{P. formosum} and \textit{P. longisetum} (chapter 3) do support a division of the genus \textit{Polytrichum} s.l. into the genera \textit{Polytrichum s.s.} and \textit{Polytrichastrum} (Smith, 1971). Moreover, phylogenetic trees constructed in the chapters 2 & 4 show, in addition to those published in other recent studies on the Polytrichaceae using morphological characters as well as allozyme electrophoresis and DNA sequencing (Hyvönen et al., 1998; Derda et al., 1999), that species belonging to either of these two genera cluster in different groups. However, as the level of divergence between the three clusters \textit{P. piliferum} and \textit{P. juniperinum}, \textit{P. commune} and \textit{P. uliginosum}, and \textit{P. formosum} and \textit{P. longisetum} is approximately equally high, this indicates that if the division of \textit{Polytrichum s.s.} and \textit{Polytrichastrum} is accepted then the taxonomic status of the section Juniperifolia of the genus \textit{Polytrichum} should also be elevated to the level of a separate genus as well (chapter 4; Derda et al., 1999).

In addition to elucidating taxonomic relationships between bryophyte taxa, studies using molecular markers also have given better insight into the phylogenetic relationships between diploid bryophyte species and their tentative haploid progenitors. For example, many diploid species that, based on their morphology, have been thought to be autodiploids, have recently been shown to be allodiploid species (Wyatt et al., 1992; Wyatt et al., 1993a,b; Cronberg, 1996a; Wyatt & Odrzykoski, 1998). In this study, both the chromosome number of \(2n=14\) (the basic chromosome number for the Polytrichaceae is regarded to be \(n=7\); Ramsay, 1983), as well as the fixed heterozygous banding patterns observed for both allozyme and microsatellite loci indisputably show that \textit{P. longisetum} is also an allodiploid species (chapter 3). Comparison of the diploid allozyme and microsatellite banding patterns of \textit{P. longisetum} with those observed for the most closely related extant \textit{Polytrichum} species indicates that \textit{P. formosum}, or one of its recent ancestors, is one of the two haploid progenitor species of \textit{P. longisetum}, and that the second progenitor species may be extinct (chapter 3). These findings are especially interesting from a taxonomic point of view, because (i) \textit{P. formosum} has also been mentioned as a possible progenitor species of three other allodiploid \textit{Polytrichum} s.l. species: \textit{P. ohioense}, \textit{P. sexangulare} and \textit{P. pallidisetum} (D erda et al. 1999), and (ii) these three allodiploid \textit{Polytrichum} s.l. species together with \textit{P. formosum} and \textit{P. longisetum}
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summarising Discussion
have all been assigned to the genus Polytrichastrum by Smith (1971). This suggests that
the genus Polytrichastrum is comprised of mainly allodiploid species and their haploid
progenitors.

Based on the results obtained so far, it is expected that the application of these
molecular markers in the future, in addition to morphological characters, will provide us
with a much better understanding of the phylogenetic relationships between the taxa of the
moss genus Polytrichum.

Evolutionary Rate
Allozyme data have shown that in general bryophyte species are genetically highly
diverged, as Nei's (1987) genetic identities (I) between congeneric moss species ranged
from I = 0.21-0.63 for several different moss genera (see Table 2.5 of chapter 2, and
chapter 4). These identities are all lower than the average genetic identity observed for
congeneric species of several different vascular plant genera (I=0.79; Crawford, 1983).
Moreover, the genetic identity observed between the closely related taxa P. commune and
P. uliginosum (I = 0.61), which traditionally have been regarded as varieties of one species
but in this thesis have been shown to be distinct species (chapter 2), is even lower than the
average identity observed for congeneric vascular plants (I=0.79; Crawford, 1983). This
indicates that, in contrast to what previously has been assumed on basis of observed
differences in morphology (Anderson, 1963; Longton, 1976; Mishler, 1988; Wyatt et al.,
1989b; Emmos, 1990), the evolutionary rate of mosses, at least at the protein level, has not
been constrained by lack of genetic variation (chapter 4). Alternatively, it might be that
the evolutionary origin of most of the examined moss species mentioned in Table 2.5
(chapter 2) is much earlier than that of the examined vascular plant species reviewed by
Crawford (1983). As such, these moss species may have been reproductively isolated and,
consequently, may have genetically diverged over a much longer time than the examined
vascular plant species. This may have resulted in these much lower levels of genetic
identity between the studied congeneric bryophyte species.

The Polytrichum species examined in this study are genetically diverged to a greater
extent (I = 0.22) than most other congeneric moss species studied so far (Table 2.5 of
chapter 2; chapter 4). The high level of divergence between these species is also
illustrated by the relatively low number of microsatellite primersets that have been
developed specifically for P. formosum (chapter 5), which showed also successful
amplification in related Polytrichum species (Table 3.3 of chapter 3). This contrasts to the
higher levels of across-species applicability of microsatellite primers observed for other
related plant species groups (chapter 3). As the Polytrichales are generally regarded as an
ancient or “primitive” group of species (Crum, 1976; Wyatt, 1982), and evolutionary older
than most other groups of mosses (Mishler & Churchill, 1984), the origin of the examined
Polytrichum species may date back to before the origin of most of the other examined
moss species. This possibly explains the much lower genetic identities observed between
the Polytrichum species compared to those observed between other congeneric moss
species. However, part of the differences in mean genetic identity between the different
moss genera may also be explained by differences in the methodology of allozyme
electrophoresis used by the different investigators. This suggestion is supported by the
large difference between the genetic identity described in this study ($I = 0.22$; chapter 4) and that reported by Derda et al. (1999), who observed an average genetic identity of $I = 0.68$ between mainly American populations of *P. commune*, *P. formosum*, *P. juniperinum* and *P. piliferum*, species that have also been examined in this study. A counter-argument is that the mean genetic identities observed between congeneric species of the genera *Racopilum* and *Plagiothecium* (De Vries et al., 1983, 1989; Hofman, 1991), showing, on average, much higher identities than observed for *Polytrichum* (chapter 2 & 4), were determined in the same laboratory using the same procedures and methodology for protein electrophoresis as applied in this study.

**Differences in Level of Genetic Variation among Species**

The *Polytrichum* species examined in this study, and more specifically *P. commune*, *P. uliginosum* and *P. formosum*, show lower levels of allozyme variation ($H_r = 0.025-0.166$; chapter 4) than those reported for most other moss species and vascular plants (Wyatt et al., 1989b; Hamrick & Godt, 1990; Stoneburner et al., 1991). Nevertheless, comparably low levels of gene diversity have also been observed for a number of other moss species (chapters 4 & 8; Hofman, 1991; Wyatt, 1992). As relatively low levels of genetic variability have also been reported for North American populations of these *Polytrichum* species (Wyatt & Derda, 1997; Derda & Wyatt, 1999a,b), it can be concluded that *Polytrichum* species seem to be among the least genetically variable moss species examined so far (chapter 4).

Conspicuous differences in the mean level of gene diversity exist among congeneric moss species (e.g. De Vries et al., 1983, 1989; Hofman, 1991; Wyatt, 1992; Wyatt & Derda, 1997; Cronberg, 1998; Shaw, 1999). Among the *Polytrichum* species examined in this study significant differences in the mean level of gene diversity have also been observed ($H_s = 0.022-0.127$; chapters 4 & 8). For vascular plants, Hamrick & Godt (1990) have shown that, among other characteristics, the breeding system has a significant effect on the level of genetic variation in natural populations. As such, it was shown that selfing plant species exhibit less genetic variation than outcrossing species (Hamrick & Godt, 1990). For bryophytes, several authors have argued that monoecious (facultatively selfing) species may exhibit lower levels of genetic variation than dioecious (obligatory outcrossing) species (Wyatt et al., 1989b; Cronberg, 1998), because of the high level of self-fertilisation in monoecious species, which is genetically equivalent to asexual reproduction (see General Introduction). Hofman (1988, 1991) and Cronberg (1998) have investigated whether differences in the breeding system of bryophytes were correlated with differences in the level of genetic variation between congeneric species of the moss genera *Plagiothecium* and *Sphagnum*, respectively. In both studies considerable differences in the level of genetic variation were observed between monoecious and dioecious species. However, in contrast to the expectation, monoecious species proved more variable than dioecious species in these studies.

As all examined *Polytrichum* species are dioecious, differences in sexuality as described above, therefore, can not account for the observed differences in genetic variation between these species (chapters 4 & 8). However, *P. piliferum* and *P. juniperinum* are generally observed in more dynamic habitats, where the turnover of genets is high, and thus might be more prone to drift or also stochastic gene flow. However, an important factor (chapter 5) that affects the rate of genetic turnover is habitat structure (Hofman, 1991). From this it has been shown that plants growing in more dynamic habitats have higher levels of genetic variation than those growing in more stable environments.

Genetic Variation

The distribution of mutations is random (Wright, 1951), and therefore it is not surprising that for most of the species examined evolutionary forces would also be important (chapter 9). From this, it is found that plants growing in more dynamic habitats have higher levels of genetic variation than those growing in more stable environments.

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Summarising Discussion

Genetic Variation among Populations within Species

The distribution of neutral genetic variation among populations is mainly the result of random genetic drift within populations, counteracted by gene flow between populations (Wright, 1951). Therefore, the level of gene flow is an important determinant of the genetic structure among populations. Additionally, historic events, as e.g. the glacial periods, have also been shown to affect the genetic structure among many plant populations to a large extent (e.g. Taberlet et al., 1998).

In bryophytes, gene flow can be mediated by asexual propagation, male gametes and spores. Generally, asexual diaspores and male gametes are thought to have a much more limited dispersal range than spores (Wyatt, 1982, 1994; Longton, 1976; Kimmerer, 1991; Newton & Mishler, 1994), and sexually produced spores, therefore, are probably the most important factor facilitating gene flow between bryophyte populations. By means of a paternity analysis of sporophytes using microsatellite markers, it is shown in chapter 7 that for P. formosum male gamete dispersal is restricted to maximally a few metres. Additionally, the absence of identical multi-locus genotypes for spatially disjunct moss cushions both within populations and from different populations (chapters 6 & 7) indicates that asexual propagation of gametophytes is also restricted to a few metres. Consequently, male gametes and asexual propagation are indeed unlikely to facilitate gene flow between populations in this and possibly (most) other Polytrichum species.

Potentially, spores are able to disperse over much larger distances (e.g. Van Zanten & Pócs, 1981) and may, therefore, facilitate gene flow between populations. Such long distance effective spore dispersal is practically impossible to measure in the field, but,
Chapter 9

under the assumption of an “island model” of migration, it can be estimated indirectly from the relationship between the level of genetic differentiation \( (F_{ST}) \) and the level of gene flow \( (Nm) \) between populations \( (F_{ST}=1/[4Nm+1], \) Wright 1951). As the examined Polytrichum populations are mainly composed of haploid populations, drift is twice as effective in these haploid moss than in diploid plant populations: \( F_{ST}=1/[2Nm+1] \) (Whitlock & McCauley 1999). This means that the level of gene flow has to be twice as high in haploid bryophyte populations compared to diploid plant populations in order to achieve the same level of population differentiation.

For most Polytrichum species (P. formosum, P. commune, P. uliginosum and P. piliferum), which have been examined for a large geographical range within Europe in this study, the low levels of genetic differentiation \( (F_{ST} \leq 0.1) \) between populations, the absence of “isolation by distance” and the lack of clustering of regional populations show that there is no clear genetic structuring within these species (chapter 4, 6 & 8). This indicates that extensive gene flow has been the most important determinant of genetic structure among populations in these species. In contrast, for P. juniperinum high levels of population differentiation \( (F_{ST} = 0.34) \) and a clear genetic structure have been observed among European populations (chapters 4 & 8). A clear bifurcation of populations in this species possibly shows the impact of the recolonisation of Europe from two different refugia after the last glacial period in combination with the low levels of gene flow between these two groups of populations on the genetic structure of this species (chapter 8). Wyatt & Derda (1997) and Derda & Wyatt (1999a,b) observed considerably higher levels of genetic differentiation among North American populations of P. commune, P. piliferum and P. juniperinum than those reported in this thesis for European populations of these species (chapter 8). These differences in the level of population differentiation between the continents for each of these species may be explained by a different impact of the glacial periods on the genetic structure of North American compared to European populations of these species (Wyatt et al., 1993b).

From the previous paragraph it is evident that extensive gene flow is an important determinant of genetic structuring within Polytrichum species. It appears that spores can effectively disperse, and prevent substantial genetic differentiation by random drift, between populations at least up to 1500 km apart (chapter 8). The fact that for microsatellites alpine populations of P. formosum were shown to be substantially differentiated both from each other and from lowland populations (chapter 8), suggests that mountains possibly act as barriers of gene flow, and reduce spore dispersal between populations to a certain extent. In addition, substantial levels of genetic differentiation between populations from different continents suggest that also extremely long distances and oceans may limit the level of gene flow considerably (chapter 8). The latter is corroborated by a study on Plagiomnium ellipticum (Wyatt et al., 1992), where relatively higher levels of genetic differentiation were observed between populations from the North American and European continent, than between populations within each of these continents. Nevertheless, the level of differentiation between continentally disjunct bryophyte populations is generally much lower than that observed for continentally disjunct vascular plant populations (Wyatt, 1994), showing that dispersal of spores is facilitating gene flow among continents more effectively than seed dispersal.
Summarising Discussion

Genetic Variation within Populations

Since mosses are able to reproduce asexually quite easily (Anderson, 1963; Longton, 1976; Mishler, 1988; During, 1990), bryophyte populations could be expected to possess a genetic structure considerably different from that of obligatorily sexually reproducing plants (Murawski & Hamrick, 1990; Shapcott, 1995; McFadden, 1997). For example, populations of clonally reproducing bryophyte species may be comprised of only a few large genets (individuals). In contrast, high levels of genotypic diversity (i.e. number of different multi-locus genotypes = genets) were observed within populations and within large moss-cushions of *P. formosum* (chapters 6 & 7). This shows that in *P. formosum* sexual reproduction is a much more important determinant of genetic structure at these spatial scales than previously thought (chapters 6 & 7). On the other hand, identical multi-locus genotypes were also frequently observed, but only for spatially clustered shoots, showing that in this species asexual reproduction occurs mainly through branching and horizontal proliferation of gametophytes through underground rhizomes (i.e. vegetative growth of genets; chapter 7). Consequently, asexual reproduction seems to be important only at a small, very local scale in *P. formosum*.

The importance of sexual reproduction in *P. formosum* seems to contradict the rarity of observations of recruitment of new genets from spores in natural bryophyte populations (Hobbs & Pritchard, 1987; Miles & Longton, 1987, 1990; Innes, 1990). However, the infrequent observation of recruitment from spores might be due to the difficulty of demonstrating its occurrence (Newton & Mishler, 1994). On the other hand, if recruitment from spores is indeed rare, extreme longevity of genets, due to extensive clonal growth, may over long time periods allow for accumulation of genets in populations resulting in high levels of genotypic diversity within these populations (e.g. Sebens & Thorne, 1985; During & Van Tooren, 1987; Eriksson & Fröborg, 1996). Alternatively, as the *P. formosum* population examined in chapter 7 was growing under beeches planted in 1923, large scale disturbance of a then present diaspore bank by this plantation, in combination with small scale disturbance of the forest soil every 5 year, due to cutting of the branches of these trees, could also account for the relatively high genotypic diversity. Similarly, Hofman (1991) and Cronberg (1996) argued that disturbance of the soil, which is thought to be important for diaspore germination (During, 1997), also resulted in increased levels of genotypic diversity in *Plagiothecium undulatum* and *Sphagnum capillifolium* at particular spots in the population.

Concluding Remarks

Although *Polytrichum* species show on average lower levels of genetic variation than most other bryophyte species (this thesis; Wyatt & Derda, 1997), in general, mosses seem, despite their predominantly haploid life cycle, not to exhibit lower levels of genetic variation than diploid vascular plants (Wyatt *et al.*, 1989b; Hamrick & Godt, 1990; Stoneburn *et al.*, 1991). In addition, the levels of genetic variation observed in (facultatively) asexually reproducing plants, including bryophytes, are, in general, comparable to those of sexually reproducing plants (Ellstrand & Roose, 1987; Hamrick & Godt, 1990; Widén & Widén, 1994). This indicates that neither a predominantly haploid
life cycle nor the ability to reproduce asexually does result in reduced levels of genetic variability in bryophyte species. However, the high genotypic diversity observed for *P. formosum* indicates that sexual reproduction possibly outweighs asexual reproduction in the long run, and is a much more important determinant of the genetic structure of bryophyte populations than previously thought. Therefore, in addition to studies that monitor populations over many years to get estimates of genet recruitment frequencies and genet turnover, more detailed studies on the genetic structure of populations involving species with different reproductive systems (Hofman, 1991; Cronberg, 1998) and species with differences in the predominance of asexual and sexual reproduction (this study; Shaw, 1999) are required to unravel the effects of different genetic systems and life history characteristics on the level of genetic variation within bryophyte species. Such studies will be greatly facilitated by the application of modern molecular techniques.