Phylogeny of Eurasian Stipeae, genetic structure and seed germination of *Stipa spp.* in Jordan

Dissertation

zur Erlangung des akademischen Grades
doctor rerum naturalium
(Dr. rer. nat)

vorgelegt der

Naturwissenschaftlichen Fakultät I Biowissenschaften
der Martin-Luther-Universität Halle-Wittenberg

von

Herrn Hassan Refai Hamasha

geb. am: 28.03.1977 in: Amman (Jordan)

Gutachter /in

1. Prof. Dr. Isabell Hensen, Martin-Luther-Universität, Halle-Wittenberg, Germany
2. Prof. Dr. Ahmad El-oqlah, Yarmouk University, Irbid, Jordan.
3. Prof. Dr. Christoph Oberprieler, Universität Regensburg, Regensburg, Germany

Halle (Saale), verteidigt am 01. Juni 2010

’’رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ“

القرآن الكريم: آية (19) سورة النمل

"O my Lord! so order me that I may be grateful for Thy favours, which thou hast bestowed on me and on my parents, and that I may work the righteousness that will please Thee: And admit me, by Thy Grace, to the ranks of Thy righteous Servants."

Al-Quran Al-Kareem: Al Naml (19)

Contents

Summary

Jordan is of great interest to vegetation ecology as it has a unique topography that varies markedly over very short distances which has led to the formation of several distinct bioclimatic regions. The genetic diversity, population structure and seed germination were studied for four Jordanian *Stipa* species occurring along a common environmental gradient spanning arid habitats with highly variable and unpredictable rainfall to Mediterranean habitats with less variable and more predictable rainfall. The central objective of the present thesis was to test whether the environmental gradient affects genetic diversity, population structure and seed germination of Jordanian *Stipa* species in a similar or distinct manner. As hitherto no molecular study of the Old World Stipeae had been conducted, a study into the phylogeny of Eurasian Stipeae species, including the Jordanian *Stipa* species, was undertaken. In general, genetic diversity correlated positively with population size, whereas both genetic diversity and seed germination percentage correlated negatively with rainfall. Our results showed, however, that the four Jordanian *Stipa* species respond differently, along the same ecogeographical gradient. In terms of their population genetic characteristics, they can be divided into two major groups. The first group, which is composed of the ruderal species *S. capensis* and *S. parviflora*, exhibited higher genetic diversity than the second group of closely related semi-desert species *S. arabica* and *S. lagascae*, but genetic differentiation was stronger in the semi-desert species than in the ruderals. The genetic diversity of the ruderals was mostly affected by population size while the genetic diversity of the semi-desert species was more strongly affected by the environmental gradients of temperature and precipitation. Principal coordinate analysis (PCoA) and STRUCTURE analysis showed that the *Stipa* populations were grouped ecogeographically, because those sharing the same climatic and edaphic environment grouped together in the same cluster irrespective of the geographic distances between them. The Saharan Mediterranean populations of Jordanian *Stipa* were clearly separated from the semiarid and arid Mediterranean populations in all studied species, with a more pronounced differentitation being recorded for the semi-desert species as the arid Mediterranean populations were also separated from the semiarid populations. The variations in seed germination among populations were species-specific; populations from arid and Saharan regions showed higher germination percentages than those from semiarid regions, but differences were not significant. The most suitable temperature levels for germination also varied among species and ranged from low (8/4°C and 20/10°C) for *S. lagascae*, to high (20/10°C and 32/20°C) for *S. capensis*, while *S. arabica* and *S. parviflora* germinated equally well at all three temperature regimes. Moreover, I found that the four Jordanian *Stipa* species belong to two different monophyletic lineages as the phylogeny of Eurasian Stipeae revealed five highly supported monophyletic lineages, namely: 1) the narrowly defined *Stipa* core clade, which includes our semi-desert Jordanian *Stipa* species (*S. arabica* and *S. lagascae*) and covers the Eurasian lineage extending into Africa north of the Sahara Desert, 2) the Old World *Piptatherum* clade, 3) a Himalayan to E Asian clade, 4) the single species *Achnatherum splendens*, and 5) the large "Transcontinental Stipeae Clade" encompassing Eurasian, African, American, and Australian lineages and which includes several lineages of Eurasian Stipeae other than the *Stipa* core, i.e. the genera *Aristella*, *Celtica*, *Oloptum* gen. nov., *Stipella* stat. et. gen. nov. (including our ruderal Jordanian *Stipa* species (*Stipella capensis* and *Stipella parviflora*); and any new combinations that are made), species of *Achnatherum*, and the species-rich lineages of *Nassella*/*Jarava* in America and of *Austrostipa* in Australia. *Oloptum*, gen. nov., was described and several combinations have been described.

In conclusion, our phylogenetic study allows for the firm circumscription of a narrowly defined *Stipa* core clade and reveals the origin of the typical American and Australian Stipeae from the separate "Transcontinental Stipeae Clade". The genetic diversity of Jordanian *Stipa* populations increased with environmental stress, while the population structure is a result of both edaphic and climatic factors. However, as AFLP is considered to be selectively neutral thereby making it difficult to conclude to what degree selection pressures affect the pattern of genetic variation within plant species. Therefore, further studies with quantitative genetic methods such as quantitative trait locus (QTL) mapping studies are recommended. Moreover, seed germination showed flexibility among temperatures and populations and this could be interpreted as an efficient survival strategy. The thesis results are valuable for conservation and management in Jordan and, where e.g. artificial reseeding is necessary, differences among species as well as among different seed provenances should be taken into account.

Jordan belongs to the Mediteranean region that is considered a hot spot of biodiversity (Myers *et al.* 2000). Although Jordan is a small country, its vascular flora has more than 2500 species, which represents 1% of the total world flora, of which 100 are endemic (GCEP 2000). The presence of unusual ecological conditions in Jordan and the variation in climate, edaphic conditions and topography as well as the long history of human activity in the area have led to a wide diversity in ecological habitat and flora (Al-Eisawi 1996). Such contrasting climates are expected to affect the demographic and genetic performance of Jordanian plant species. *Stipa* species have a broad geographical distribution in Jordan and constitute an excellent model to expand our general knowledge of population dynamics. Therefore, the main aim of this thesis was to study the genetic diversity, genetic structure and seed germination of four Jordanian *Stipa* species differing in their life-form, ecology, and geographical distribution. As steep environmental gradients can strongly affect the genetic structure of plant populations, the extent to which closely related species respond similarly or differently along the same ecogeographical gradient was tested for the present thesis.

Environmental variability and genetic diversity

Genetic diversity is a fundamental feature of species, populations and ecosystems, because it represents the evolutionary potential to survive in a changing environment. The evolution of genetic differentiation is a non-random and structured process at both the molecular and organism levels and across all geographical scales (Nevo 2001; Kis-Papo *et al.* 2003). In general, genetic diversity arises from mutation or may be added to a population by gene flow, whereas genetic drift and directional selection may eliminate it. Genetic diversity and population genetic structure are affected by various evolutionary forces such as mutation, mating system, meiotic behaviour, geographical range, genetic drift due to finite population size (e.g. following founder effects), gene flow (e.g. pollen/seed dispersal) and selection (Hamrick & Godt 1990; Linhart & Grant 1996; Hedrick 1998). Demographic factors such as population size fluctuations and gene flow between populations modulate genome-wide levels and are expected to have a direct impact on the genetic variation among populations (Lawton-Rauh *et al*. 2007). Moreover, human induced habitat fragmentation, which divides large and continuous populations into smaller populations, have a major impact on the strength of genetic drift as well as on gene flow (Pluess & Stoecklin 2004; Hensen & Oberprieler 2005). These evolutionary and anthropogenic forces are of great interest to ecological and population genetic studies (Hedrick 1998; Nevo *et al.* 2002).

Environmental variability, as well as ecological diversification between habitats, is crucial for the maintenance of genomic diversity due to the evolutionary forces that shape the genetic structure and differentiation between local populations (Linhart & Grant 1996; Gram & Sork 2001). Genomic diversity is assumed to be positively associated with abiotic and biotic environmental heterogeneity as well as stress as different genotypes display varying fitness in differing environments and under different types of stress (Nevo 2001). Unstable environmental and demographic stochasticity lead to significant fluctuations in population sizes and in some instances extinction (Boyce 1992; Østergaard *et al*. 2003). However, when an environment is heterogeneous and genetic variation within plant populations is spatially structured, it may either indicate habitat selection or be a result of the combined effects of restricted gene flow, inbreeding, and genetic drift (Heywood 1991; Linhart & Grant 1996). In addition, demographic processes may produce patterns of genetic variation that resemble the outcome of natural selection (Nielsen 2001; Przeworski 2002) and inferences of natural selection are confounded by population demographic history.

According to the niche-width variation hypothesis (Van Valen 1965) and the environmental theory of genetic diversity (Nevo 1988, 1998), populations from spatiotemporally variable and ecologically more stressful habitats are expected to display higher genetic polymorphism. However, the genetic structure of ecologically subdivided populations could be explained by natural selection (Nevo *et al*. 1998) and could also be explained by restrictions to gene flow independent from selection, by metapopulation dynamics, or by historical factors (Comes & Abbott 1999). As such, the hypothesis of this thesis is that environmental variations as well as demographic processes have a strong impact on the genetic diversity and population structure of *Stipa* species in Jordan. To test this hypothesis, amplified fragment length polymorphism (AFLP) was used, as it is a molecular marker known to provide information on genome-wide variation, genetic structure and gene flow both within and between species (Vos *et al*. 1995; Jiang *et al*. 2004).

Seed germination of *Stipa* species

Stipa-species grow in semiarid and arid environments in a large proportion of the Eurasian zonal vegetation, suggesting that they are valuable in controlling desertification (Lavernko & Karamysheva 1993). Seed provenance and genotypic diversity may have strong effects on the performance of populations introduced for biodiversity restoration or habitat creation (Bischoff *et al*. *in press*), and the use of local seed provenances is recommended because they are thought to be better adapted to local habitat conditions (Bischoff *et al*. 2006; Bischoff *et al*. *in press*). Differences among provenances, particularly in seed dormancy and germination, are not only affected by the current environmental conditions but also by conditions experienced by mother plants in the previous generation as the differences in germination can be inflated by heterogeneous environmental conditions among sites at which seeds were collected (Gutterman 1994; Baskin & Baskin 1998). In addition, germination behaviour may vary greatly within a single species from one population to another, from year to year and among individuals (Gasque & García-Fayos 2003).

Plants have evolved various life-history strategies in response to the spatiotemporal variation caused by the variability in abiotic and biotic factors (Silvertown & Charlesworth 2001; Ehrlén 2002). In arid environments, which are characterized by highly variable and unpredictable climates, seed germination, particularly of annual plants, greatly reduces the variance in fitness across years. The mechanism allows buffering against reproductive failure by spreading seed germination over several years (Philippi & Seger 1989; Philippi 1993). Seed germination rate is also an important trait in plants adapted to arid environments, as subsequent fitness directly depends on the arrival of rains and the rapid germination of seeds (Gutterman 1994; Whitford 2002). Plants growing under more mesic and predictable Mediterranean conditions are assumed to exhibit higher germination percentages due to increasing mean annual rainfall and its predictability (Petru° & Tielbörger 2008). The onset of flowering also varies among different ecotypes and tends to be earlier in the arid ecotype than in conspecific plants growing under more humid conditions (Volis *et al*. 2007). No reports are available on the germination of *Stipa* species and their populations from Jordan. Therefore, seed germination patterns of *Stipa* species were studied because in semiarid and arid regions of the world, such as Jordan, the low amount of precipitation and its irregular distribution in time make living conditions for plants extremely harsh. Their reproductive success crucially depends on whether their seeds germinate in the right place and at the right time (Gutterman 1994).

Phylogeny of Eurasian Stipeae species

The taxonomy of Old World Stipeae has been studied to date mainly from a morphological and anatomical perspective. Based on these data, the four Jordanian *Stipa* species are placed in two different sections within the genus (Freitag 1985). *Stipa capensis* and *S. parviflora* belong to the section S*tipella*, which is characterized by a tendency toward reduction of the upper lodicules; whereas *S. arabica* and *S. lagascae* belong to the section *Barbatae*, which is primarily characterised by the ovary carrying 3 or 4 styles and stigma (Freitag 1985). Delimitation of the genera and assignment of the species in Stipeae are still in a state of flux. As there was no molecular study of the Old World Stipeae, this thesis includes a study of the phylogeny of Eurasian Stipeae species including the Jordanian *Stipa* species.

Stipeae (Pooideae) are a cosmopolitan grass tribe of c. 400 species with a worldwide distribution occupying a wide ecological range that includes arid, semiarid, semihumid and humid habitats at varying elevations from 0 to 5000 m (Barkworth & Torres 2001; GPWG 2001). The tribe is characterised by having single-flowered spikelets with terminally-awned lemmas, florets with two or three linear lodicules and small-sized chromosomes with a base number of x = 10-12 (Romaschenko *et al*. 2008). The largest and most widespread genus is *Stipa* with around 140 species in its current circumscription (Barkworth *et al*. 2008). Stipeae have been a constant subject of attention by systematists, who have evaluated relationships in this group for hundreds of years on the basis of morphological, anatomical, and, more recently, molecular data, which have been used to describe new genera and emend generic limits (eg., Cialdella *et al*. 2007; Romaschenko *et al*. 2007, 2008; Barkworth *et al*. 2008). Nowadays there are 21 accepted genera of Stipeae (Soreng *et al*. 2003, 2008); *Achnatherum* P. Beauv., *Aciachne* Benth., *Ampelodesmos* Link, *Amelichloa* Arriaga & Barkworth, *Anemanthele* Veldkamp, *Austrostipa* S.W.L. Jacobs & J. Everett, *Celtica* F.M. Vázquez & Barkworth, *Hesperostipa* (M.K. Elias) Barkworth, *Jarava* Ruiz & Pav., *Macrochloa* Kunth, *Nassella* (Trin.), *Ortachne* Nees ex Steud., *Orthoraphium* Nees, *Oryzopsis* Michx., *Pappostipa* (Speg.) Romasch., P.M. Peterson & Soreng, *Piptatherum* P. Beauv., *Piptochaetium* J. Presl, *Psammochloa* Hitchc., *Ptilagrostis* Griseb., *Stipa* L., and *Trikeraia* Bor.

Historically, delimitation of genera within the Old World Stipeae was based on a broad concept of the genus *Stipa* L.. Bentham (1882) reduced what we now recognize as the Stipeae to two genera, *Stipa* and *Oryzopsis*. Hitchcock (1935, 1951) accepted three genera in North America: *Oryzopsis* Michx., *Piptochaetium* Presl., and *Stipa.* In South American Stipeae, Spegazzini (1901) recognized only *Aciachne* Benth., *Oryzopsis* and *Stipa.* This thesis concentrates on the Eurasian species only, while a more complete summary of the pre-molecular-era taxonomy can be found in Barkworth (1990). Freitag (1985) adopted a broad concept of the genus *Stipa* itself, and classified Asian species of *Stipa* by treating *Achnatherum* and *Ptilagrostis* as sections, which relocates the problematic circumscription to a lower taxonomic level, and only *Piptatherum* was treated as a separate genus. However, Russian and Chinese authors have generally recognized *Achnatherum* as a separate genus (Tzvelev 1976; Lu & Kuo 1987). This separation was also supported by Barkworth and Everett (1987) and Barkworth (1993).

The genera *Stipa*, *Achnatherum*, *Piptatherum* and *Ptilagrostis* were originally described from Asian species but they have been enlarged to encompass American species (e.g., Barkworth 1993). However, recent molecular phylogenetic studies confirmed that several taxa were not well defined, i.e., numerous new and as yet unnamed monophyletic lineages were revealed, while other more traditional genera seemed to be highly poly- or paraphyletic (Jacobs *et al*. 2000, 2007; Barkworth *et al*. 2008; Romaschenko *et al*. 2007, 2008; Cialdella *et al*. 2007). The American taxa were partly resolved as monophyletic, e.g., *Pappostipa* (Romaschenko *et al*. 2008) or *Piptochaetium* and *Hesperostipa* (Barkworth *et al*. 2008), but the circumscription of *Jarava* and *Nassella* remains doubtful. The 34 Australian species analysed to date and *Anemanthele* from New Zealand were almost monophyletic with very few exceptions (Jacobs *et al*. 2007). Although only a few Eurasian *Stipa* s. str. species were included in their molecular analyses (Jacobs *et al*. 2007; Barkworth *et al*. 2008), the monophyly of both Eurasian groups was not proven. In common with *Stipa* s. str., the Eurasian taxa of *Piptatherum*, *Ptilagrostis* and *Achnatherum* were polyphyletic and were placed in two different tree regions (Barkworth *et al*. 2008). In summary, the phylogeny of Eurasian taxa has as yet been neglected, and very few of the c. 160 Stipeae species from Eurasia have been evaluated despite the molecular work that was done on American and southern hemispherical lineages.

Thesis structure and objectives

This thesis consists of four chapters. This first chapter introduces the scientific background upon which hypotheses and experiments are based, and presents an overview of the area and species studied. The following three major chapters, which can be read independently, are organized much like journal publications and contain an introduction followed by methods, results, discussion and references.

In **chapter 2**, the genetic diversity and population structure of four Jordanian *Stipa* species (*S. capensis*, *S. parviflora*, *S. arabica* and *S. lagascae*) along the same ecogeographical gradient using AFLP markers is discussed. The following questions are addressed: Is genetic diversity within populations related to environmental variables and population size? Do patterns differ among *Stipa* species? How is genetic variation distributed among and within populations of the four *Stipa* species? Do patterns of population structure provide evidence of species-specific ecogeographical structuring?

Chapter 3 deals with the seed germination of the same four Jordanian *Stipa* species distributed throughout differing phytogeographical regions. The variation in seed germination was studied among populations under laboratory conditions and with various temperature regimes. Specifically, the following questions are addressed: Do the provenances of the four test species differ in germination percentage with respect to their bioclimatic region? What are the optimum temperatures for germination for each species?

Chapter 4 examines the phylogeny of 109 new ingroup taxa with a focus on Old World Stipeae (including an extensive outgroup sampling) based on chloroplast and nuclear ribosomal DNA sequences. The chapter aims to investigate the ''core'' of *Stipa* and provide evidence on the origin of American and Australian lineages with a view to presenting a phylogenetic hypothesis for as many Eurasian Stipeae as possible based on nuclear and chloroplast sequence data. The hypothesis is subsequently employed to clarify taxonomic boundaries within the Stipeae and to resolve biogeographical questions.

The study area

Jordan is classified as an arid area and is situated at the transition zone between arid and semiarid bioclimates. It acts as a faunal and floral bridge between the continents of Asia, Africa and Europe and harbours a strong environmental gradient from Mediterranean to desert climate (GCEP 2000; Danin 1995; Dahamsheh & Aksoy 2007). Biogeographically, Jordan consists of nine bioclimatic regions (Al-Eisawi 1985, 1996) that belong to four major phytogeographical regions; these include the Mediterranean, Irano-Turanian, Saharo-Arabian regions and Sudanian regions, which were formed due to a range of ecological factors (Zohary 1973; Al-Eisawi 1985, 1996; GCEP 2000; Al-Quran 2008). The occurrence of four regions and the presence of contrasting ecological conditions that vary sharply and suddenly over very short distances in a small country (90,000 km^2) has a great influence on the plant life and diversity (GCEP 2000; Freiwan & Kadioglu 2008).

The climate in Jordan varies from one region to another, and the topography of the land is the main factor controlling the spatial distribution of rainfall and temperature (Freiwan

& Kadioglu 2008). Climatically, most of Jordan can be classified as semi-desert, with only the western highlands being characterized by a Mediterranean climate. Over 90% of the land area has an annual rainfall of less than 200 mm, while only 2% has more than 350 mm (Dahamsheh & Aksoy 2007). Generally, rainfall decreases and temperature increases southwards and eastwards, with much spatiotemporal variation: from 600 mm of precipitation in Ajloun to 300 mm in Moav to 100-300 mm in Edom, and 50 mm in the Southern and eastern desert (Nevo *et al*. 2000; Dahamsheh & Aksoy 2007). In addition, the rainfall is irregular and of uneven distribution, with large variability between and within the regions.

Jordanian *Stipa* species

Six *Stipa* species are recorded from Jordan, namely: *Stipa arabica* Trin. & Rupr., *S. capensis* Thunb., *S. parviflora* Desf., *S. lagascae* R. & Sch., *S. hohenackeriana* Trin. & Rubr. and *S. bromoides* L.. *S. capensis* Thunb. is the only annual grass while all others are perennials (Zohary 1962; GCEP 2000). Species selection was based on occurrence in at least three different ecogeographical regions. During the field survey, the abundance of *Stipa* species in Jordan was found to decrease from Mediterranean to desert environments; however, population density in the arid and Saharan regions within the wadies (=Arabic for ephemeral river valley) can be as high as in semiarid regions (HR Hamasha, pers. obs.). In addition, *S. bromoides* and *S. hohenackeriana* were found to be rare and there were insufficient populations to facilitate the study of their genetic structure. Therefore, four *Stipa* species differing in life form, ecology and geographical distribution were studied. These four *Stipa* species also differ in their morphological classification and chromosome number. The first two are ruderal species that have very similar climatological requirements and distribution ranges; *S. capensis* and *S. parviflora* have 2n = 34, 36 and 2n = 28, 44, respectively and belong to *Stipa* Section *Stipella* Tzvelev in the sense of Freitag. On the other hand, chromosome number is 2n = 44 in the other two semi-desert species *S. arabica* and *S. lagascae*, which both belong to the Section *Barbatae* Junge emend. Freitag (Freitag 1985; Sheidai *et al*. 2006). *Stipa* species are pollinated by wind and, according to Ponomarev (1961), they are facultatively cleistogamous producing both chasmogamous and cleistogamous flowers. Flowering and seed dispersal of all study species usually takes place between March and June (Zohary 1962).

References

Al-Eisawi D (1996) *Vegetation of Jordan*. UNESCO-Cairo Office. 266p.

Al-Eisawi DM (1985) Vegetation in Jordan. In: *Studies in the history and archeology of Jordan* (ed. Hadidi A), pp. 45–57. Department of Antiquities, Hashemite Kingdom of Jordan and London. Amman, Routledge and Kegan Paul.

Al-Quran S (2008) Comparison study of natural vegetation structure between Ajlun and Tafileh woodlands in Jordan. *Journal of Natural Products*, **1**, 146–55.

Barkworth ME (1990) *Nassella* (Gramineae: Stipeae): revised interpretation and nomenclatural changes. *Taxon*, **39**, 597–614.

Barkworth ME (1993) North American Stipeae (Gramineae): taxonomic changes and other comments. *Phytologia*, **74**, 1–25.

Barkworth ME, Everett J (1987) Evolution in the Stipeae: Identification and relationships of its monophyletic taxa. In: *Grass systematics and evolution* (eds. Soderstrom TR, Hilu KW, Campbell CS, Barkworth ME), pp. 251–264. Smithsonian Institution Press, Washington, USA.

Barkworth ME, Arriaga MO, Smith JF, *et al*. (2008) Molecules and morphology in South American Stipeae (Poaceae). *Systematic Botany*, **33**, 719–731.

Barkworth ME, Torres MA (2001) Distribution and diagnostic characters of *Nassella* (Poaceae: Stipeae). *Taxon*, **50**, 439−468.

Baskin JM, Baskin CC (1998) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. San Diego: Academic Press.

Bentham G (1882) Notes on Gramineae. *Botanical Journal of the Linnean Society*, **18,** 14–134.

Bischoff A, Steinger T, Müller-Schärer H (*in press*) The Importance of Plant Provenance and Genotypic Diversity of Seed Material Used for Ecological Restoration Published in "Restoration Ecology: doi:10.1111/j.1526-100X.2008.00454.x, 2009"

Bischoff A, Vonlanthen B, Steinger T, Müller-Schärer H (2006) Seed provenance matters – effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology*, **7**, 347–359.

Boyce MS (1992) Population viability analysis. *Annual Review of Ecology and Systematics*, **23**, 481–506.

Cialdella AM, Guissani LM, Aagesen L, Zuloaga FO, Morrone O (2007) A phylogeny of *Piptochaetium* (Poaceae: Pooideae: Stipeae) and related genera based on a combined analysis including *trnL–F*, *rpl16*, and morphology. *Systematic Botany*, **32**, 545–559.

Comes HP, Abbott RJ (1999) Population genetic structure and gene flow across arid versus mesic environments: a comparative study of two parapatric *Senecio* species from the Near East. *Evolution*, **53**, 36–54.

Dahamsheh A, Aksoy H (2007) Structural characteristics of annual precipitation data in Jordan. *Theoretical and Applied Climatology*, **88**(3-4), 201–212.

Danin A (1995) Man and the natural environment. In: *The Archaeology of Society in the Holy Land*. (eds Levy TE), pp. 24–39. Leicester University Press, London.

Ehrlén J (2002) Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). *Perspectives in Plant Ecology Evolution and Systematics*, **5**, 145–163.

Freitag H (1985) The genus *Stipa* (Gramineae) in southwest and south Asia. *Notes from the Royal Botanical Garden*, *Edinburgh*, **42**, 355–489.

Freiwan M, Kadioglu M (2008) Spatial and temporal analysis of climatological data in Jordan. *International Journal of Climatology*, **28**, 521–535.

Gasque M, García-Fayos P (2003) Seed dormancy and longevity in *Stipa tenacissima* (L.) Poaceae. *Plant Ecology*, **168**: 279–290

GCEP (General Corporation for Environment Protection, Jordan) (2000) Jordan Ecology, Ecosystems and Habitats: Plant Taxonomy. United Nations Environment Programme, Amman, Jordan.

Gram WK, Sork VL (2001) Association between environmental and genetic heterogeneity in forest tree populations. *Ecology*, **82**, 2012–1021.

Gutterman Y (1994) Seed germination in desert plants. Springer-Verlag, Heidelberg.

GWPG (Grass Phylogeny Working Group) (2001) Phylogeny and subfamilial classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden*, **88**, 373–457.

Hamrick JL, Godt MJW (1990) Allozyme diversity in plant species. In: *Plant population genetics, breeding and genetic resources* (eds Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 43–63. Sinauer Associates, Sunderland.

Hedrick PW (1998) Maintenance of genetic variation: spatial selection and self-fertilization. *American Naturalist*, **152**, 145–150.

Hensen I, Oberprieler C (2005) Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in Central Germany. *Conservation Genetics*, **6**, 63–73.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics*, **22**, 335–355.

Hitchcock AS (1935) *Stipa*, *Oryzopsis*, and *Piptochaetium*. *North America Flora*, **17**, 406−431.

Hitchcock AS (1951) Manual of Grasses of the United States. Ed. 2, revised by Chase A, Washington DC, USDA. Miscellaneous publication 200, 1−1051.

Jacobs SWL, Everett J, Barkworth ME, Hsiao C (2000) Relationships within the stipoid grasses (Gramineae). In: *Grasses, systematics and evolution*, (eds. Jacobs SWL Everett J) pp 75–82. Melbourne, Victoria, Australia: CSIRO Publishing.

Jacobs S, Bayer R, Everett J *et al*. (2007) Systematics of the tribe Stipeae using molecular data. *Aliso*, **23**, 349–361.

Jiang C-S, Jia H-S, Ma X-R, Zou D-M, Zhang Y-Z (2004) AFLP analysis of genetic variability among *Stylosanthes guianensis* accessions resistant and susceptible to the Stylo Anthracnose. *Acta Botanica Sinica*, **46**, 480–488.

Kis-Papo T, Kirzhner V, Wasser SP, Nevo E (2003) Evolution of genomic diversity and sex at extreme environments: Fungal life under hypersaline Dead Sea stress. *Proceeding of the National Academy of Science* USA, **100**, 14970–14975.

Lavrenko EM, Karamysheva ZV (1993) Steppes of the former Soviet Union. In: *Natural grasslands*. *Ecosystems of the World 8b.* (eds Coupland RT), pp. 3–60. Elsevier, Amsterdam.

Lawton-Rauh A, Robichaux RH, Purugganan MD (2007) Diversity and divergence patterns in regulatory genes suggest differential gene flow in recently derived species of the Hawaiian silversword alliance adaptive radiation (Asteraceae). *Molecular Ecology*, **16**, 3995–4013.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, **27**, 237–277.

Lu SL, Kuo PC (1987) *Achnatherum.* In: *Flora Reipublicae Popularis Sinicae*, (ed. Kuo PC), Vol **9(3)**, pp. 319–329. Science Press, Beijing.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nevo E (1988) Genetic diversity in nature: patterns and theory. *Evolutionary Biology*, **23**, 217–247.

Nevo E (1998) Molecular evolution and ecological stress at global, regional and local scales: the Israeli perspective. *Journal of Experimental Zoology*, **282,** 95–119.

Nevo E (2001) Evolution of genome-phenome diversity under environmental stress. *Proceeding of the National Academy of Science* USA, **98**, 6233 – 6240.

Nevo E, Baum BR, Beiles A, Johnson DA (1998) Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. *Genetic Resources and Crop Evolution*, **45**, 151–159.

Nevo E, Ivanitskaya E, Filippucci MG, Beiles A (2000) Speciation and adaptive radiation of subterranean mole rats, *Spalax ehrenbergi* superspecies, in Jordan. *Biological Journal of the Linnean Society*, **69**, 263–281.

Nevo E, Korol AB, Beiles A, Fahima T (2002) *Evolution of wild emmer and wheat improvement*. Springer-Verlag, Berlin, Germany.

Nielsen R (2001) Statistical tests of selective neutrality in the age of genomics. *Heredity*, **86**, 641–647.

Østergaard S, Hansen MM, Loeschcke V, Nielsen EE (2003) Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Molecular Ecology*, **12,** 3123–3135.

Petrů M, Tielbörger K (2008) Germination behaviour of annual plants under changing climatic conditions: separating local and regional environmental effects. *Oecologia*, **155**, 717–728.

Philippi T (1993) Bet-heading germination of desert annuals: variation among populations and maternal effects in *Epidium lasiocarpum*. *The American Naturalist*, **142**, 488–507.

Philippi T, Seger J (1989) Hedging one's evolutionary bets, revisited. *Trends in Ecology & Evolution*, **4**, 41–44.

Pluess AR, Stoecklin J (2004) Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany*, **91**, 2013–2021.

Ponomarev AN (1961) Klejstogomiya u Kovylej (Cleistogamy in feather grasses (*Stipa* spp.)). *Botanicheskij Zhurnal*, **9**, 1229–1235.

Przeworski M (2002) The signature of positive selection at randomly chosen loci. *Genetics*, **160**, 1179–1189.

Romaschenko K, Peterson PM, Garcia-Jacas J, Soreng RJ, Susanna A (2007) A phylogeny of Stipeae based on nuclear (ITS) sequence data. Abstract submitted to Botany 2007, available at http:// www.2007.botanyconference.org/. Last visited 10 Oct 2007.

Romaschenko K, Peterson PM, Soreng RJ *et al*. (2008) Molecular phylogenetic analysis of the American Stipeae (Poaceae) resolves *Jarava* sensu lato polyphyletic: evidence for a new genus, *Pappostipa*. *Journal of the Botanical Research Institute of Texas*, **2**, 165–192.

Sheidai M, Attaei S, Khosravi-Reineh M (2006) Cytology of some Iranian *Stipa* (Poaceae) species and populations. *Acta Botanica Croatica*, **65**, 1–11.

Silvertown J, Charlesworth D (2001) *Introduction to Plant Population Biology*, 4th edition. Blackwell Science, Oxford, UK.

Soreng RJ, Davidse G, Peterson PM *et al*. (2008) Classification of New World Grasses, available at http://mobot.mobot.org/W3T/Search/nwgclass.html.

Soreng RJ, Peterson PM, Davidse G *et al*. (2003) Catalogue of New World grasses (Poaceae): IV. subfamily Pooideae. *Smithsonian Contributions to Botany*, **48**, 1–730.

Spegazzini C (1901) Stipeae Platenses. *Anales del Museo Nacional de Montevideo*, **4**, 1–173.

Tzvelev NN (1976) *Grasses of the Soviet Union*, Vols I and II. Nauka, Leningrad. English translation 1983, Oxonion Press, New Delhi, India.

Van Valen LM (1965) Morphological variation and width of ecological niche. *American Naturalist*, **99**, 377–390.

Volis S (2007) Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology*, **21**, 381–393.

Vos P, Hogers R, Bleeker M *et al*. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Whitford WG (2002) *Ecology of Desert Systems*. Academic Press, San Diego.

Zohary D (1973) *Geobotanical Foundations of the Middle East*, Vols 1 and 2. Gustav Fischer Verlag and Swets & Zeitlinger, Stuttgart and Amsterdam.

Zohary M (1962) *Plant life of Palestine*: *Israel and Jordan*, Ronald, New York.

Chapter 2

Genetic diversity and population structure correlate with environmental factors in four *Stipa* species of Jordan, as revealed by AFLP markers

Hassan R. Hamasha, Alexander N. Schmidt-Lebuhn, Matthias Schleuning and Isabell Hensen

Submitted to *Ecography*

Abstract

Steep environmental gradients can strongly affect the genetic structure of plant populations but it is hardly known whether closely related species respond similarly or idiosyncratically to such gradients. Here we studied the genetic diversity and population structure of four Jordanian *Stipa* species along the same ecogeographical gradient using AFLP markers. The closely related ruderals *S. capensis* and *S. parviflora* exhibited higher genetic diversity than the closely related semi-desert species *S. arabica* and *S. lagascae*. In general, genetic diversity decreased with rainfall and increased with population size. Mean annual temperatures, January and August temperatures, elevation and rainfall explained more than 97% of the environmental variation among populations in all species. In *S. arabica* and *S. lagascae*, genetic diversity was mostly affected by the environmental gradient, whereas population size affected genetic diversity more in *S. capensis* and *S. parviflora*. Genetic differentiation was stronger in *S. arabica* and *S. lagascae* than in *S. capensis* and *S. parviflora* populations. Principal coordinate analysis (PCoA) and STRUCTURE analysis showed that the *Stipa* populations were grouped ecogeographically, because those sharing the same climatic and edaphic environment grouped together in the same cluster irrespective of the geographic distances between them. Consequently, the Saharan Mediterranean populations of Jordanian *Stipa* were clearly separated from the semiarid and arid Mediterranean populations. We conclude that the genetic diversity of Jordanian *Stipa* populations increases with environmental stress, while the population structure is a result of both edaphic and climatic factors. However, the steep environmental gradient in Jordan affects *Stipa* species differently.

Keywords: Ecogeographical gradients, demography, precipitation, temperature, population genetics, genetic differentiation.

Chapter 3

Seed germination of four Jordanian *Stipa* spp: differences in temperature regimes and seed provenances.

Hassan R. Hamasha and Isabell Hensen

Plant species Biology (2009), 24: 127-132.

Abstract

Stipa steppes are considered as being important model ecosystem in arid land ecology due to their wide geographical distribution and their strong association with human activities. This paper presents data on seed germination of four Jordanian *Stipa* species (*Stipa capensis*, *S. parviflora*, *S. arabica* and *S. lagascae*) which are widespread throughout various phytogeographical regions in Jordan. We studied variation in seed germination under laboratory conditions among populations and with various temperature regimes. There was significant variation in seed germination with temperature in all four species while seed provenance was significant for three out of four species. Temperature levels most suitable for germination varied from low (8/4°C and 20/10°C) for *S. lagascae*, to high (20/10°C and 32/20°C) for *S. capensis*, while *S. arabica* and *S. parviflora* germinated equally well at all three temperature regimes. Variations among populations were species-specific, but populations with the highest seed germination were always of Arid and Saharan Mediterranean origin. Seed germination was thus negatively correlated with annual precipitation. Such flexibility among temperatures and populations could be interpreted as an efficient survival strategy for species growing under unpredictable environmental conditions. Where artificial reseeding is necessary, differences among species and also among different seed provenances should thus be taken into account.

Keywords: phytogeography, restoration, *Stipa arabica*, *Stipa capensis*, *Stipa lagascae*, *Stipa parviflora*.

Chapter 4

Stipa (Poaceae) and allies in the Old World: Molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages

Hassan R. Hamasha, K. Bernhard von Hagen and Martin Röser

Plant Systematics and Evolution (Accepted pending minor revision)

Abstract

The tribe Stipeae with an estimated number of c. 400 species is part of the grass subfamily Pooideae and has near worldwide distribution. Its species are often dominant constituents of steppe vegetation and other grasslands especially in Eurasia, the Americas and Australia. The taxonomy of Old World Stipeae has been studied to date mainly from the fields of morphology and anatomy, while existing molecular phylogenetic investigations mainly dealt with New World or Australian taxa. We studied 109 new ingroup taxa with focus on Old World Stipeae (in addition with an extensive outgroup sampling) using chloroplast and nuclear ribosomal DNA sequences (3'*trnK* region, ITS1–5.8S gene–ITS2), and we discussed taxonomic key characters. Five highly supported monophyletic lineages were identified, some of which were rather unexpected: (a) the narrowly defined *Stipa* core clade, which is strictly Eurasian including Africa north of the Sahara Desert, (b) the majority of Old World *Piptatherum*, (c) a "Transcontinental Stipeae Clade" encompassing Eurasian, African, American, and Australian lineages, (d) a Himalayan to E Asian clade, and (e) the single species *Achnatherum splendens*. The large "Transcontinental Stipeae Clade" contained several lineages of Eurasian Stipeae different from the *Stipa* core (a), i.e., genera *Aristella*, *Celtica*, *Oloptum* gen. nov., *Stipella* stat. et. gen. nov., species of *Achnatherum*, and the species-rich lineages of *Nassella*/*Jarava* in America and of *Austrostipa* in Australia. In our circumscription *Ptilagrostis* was nested in (d), a clade whose internal structure (including some species of *Achnatherum* and poorly studied Himalayan species ascribed to either *Stipa* or *Orthoraphium*) remained unclear. *Oloptum*, gen. nov., is described and the following combinations are made: *Achnatherum pelliotii*, comb. nov., *Aristella keniensis*, comb. nov., *Oloptum miliaceum*, comb. nov., *Stipella*, stat. et gen. nov., *S. capensis*, comb. nov., *S. nitens*, comb. nov., *S. parviflora*, comb. nov., *S. staintonii*, comb. nov., and *S. tigrensis*, comb. nov.

Keywords: *Stipa*, *Achnatherum*, phylogeny, taxonomy, *Piptatherum*, *Stipella.*

Acknowledgments

I would like to express my hearty gratitude to my supervisor, Prof. Dr. Isabell Hensen for accepting me as a PhD candidate, her excellent supervision, valuable suggestion and support, great patience, profound expertise, endless help and encouragement during the whole doctoral period and all the manuscripts of my thesis the conduction of this work.

I am also grateful to Prof. Dr. Martin Roeser and Dr. Bernhard von Hagen for their valuable guidance, many helpful ideas and constructive discussions. I thank Dr. Alexander N. Schmidt-Lebuhn and Dr. Matthias Schleuning for their speedy help and for the many productive discussions and suggestions.

Gratitude is also extended to all other colleagues: Prof. Dr. Helge Bruelheide, Michael Beckmann, Christine Blaess, Regine Brandt, Dr. Elke Doering, Dr. Monika Partzsch, Dr. Alexandra Erfmeier, Heidi Hirsch, Sabine Kammermeier, Julia Schneider, Dr. Natalia Tkach, Beatrix Vonlanthen, Viktoria Wagner, Dr. Henrik Vonwehrden, Dr. Erik Welk, Heike Zimmermann for their readiness to help and creating a pleasant and supportive environment in the Institute of Biology/Geobotany and Botanical Garden, Martin-Luther University of Halle, Germany.

Special acknowledgements are given to the valuable assistance in the field survey of Mr. Refai Hamasha, Mr. Mohammad Hamasha and Mr. Arndt Berthold and to the valuable technical assistance in laboratory work of Mr. Sebastian Kosellek, Ms. Baerbel Hildebrandt, Ms. Birgit Mueller, Ms. Christine Voigt, and to secretarial work of Ms. Anne Piel.

I sincerely thank the DAAD (Deutscher Akademischer Austauschdienst) for grating me the scholarship for the language course and for the development of this work, respectively.

I dedicate this work to…
My beloved parents, wife, brothers and sisters who always encourage me to do this work. I can not express about my deepest thanks to my wife and parents, for their tolerance, understanding and love.

Appendices

Curriculum Vitae

Hassan Refai Hamasha

Born March 28 1977 in Amman, Jordan

Nationality: Jordanian Status: Married.

Current Address:

Institute of Biology/ Geobotany and Botanical Garden

Martin-Luther University Halle-Wittenberg, 06108 Halle, Germany

Tel. + 49 (0) 17621987631

hhamasha2000@yahoo.com hassan.hamasha@botanik.uni-halle.de

Education:

2006 - Now	PhD candidate at the Institute of Biology/Geobotany and Botanical Garden, Martin-Luther University of Halle, Germany Thesis title: **Phylogeny of Eurasian Stipeae, genetic structure and seed germination of *Stipa spp.* in Jordan** Advisor: Prof. Dr. Isabell Hensen.
1999 - 2002	M. Sc. Degree in Horticultural and Plant Protection with an average of (3.72 out of 4) Rating: "Excellent". Faculty of Graduate Studies, University of Jordan, ranked the 1st. Thesis title: "Morphological characterization and seed germination of wild almond (*Amygdalus spp.*) in Jordan".
1995 - 1999	B.Sc. Degree in Plant Production (Plant Production Department), Faculty of Agriculture, Jordan University of Science and Technology, 1999. Rating: "Very Good".
1995	General Secondary Education Certification (Tawjihi), Scientific Stream, Al Hussien Collage School.

Experiences and Skills:

Jan 2005 – Mar 2006	Research assistant in the University of Jordan on the project titled by: Deficit Irrigation of Mediterranean Agriculture Systems (DIMAS).
Jun 2002 – Dec 2004	Working with GEF/ United Nation Development Program (Conservation and Sustainable Use of Dry land Agro-Biodiversity in Jordan). Activity: Botanical survey of wild fruit trees and herbaceous vascular plants in Jordan. Collection, propagation and germination of the genetic resources of fruit trees in Jordan (*Amygdalus spp.*, *Pistacia atlantica*, *Crataegus azarolus* and *Pyrus syriaca*) and preserve them in field gene banks.
Sep 2000 – Jan 2002	Graduate teaching assistantships, faculty of graduate studies, University of Jordan.

Research Interest:

Ecological genetics, Plant population ecology, Evolutionary ecology, Biodiversity, Population and conservation genetics, Molecular phylogenetic studies in the tribe Stipeae

Languages: Arabic (native speaker), English (fluent), German (Es geht; Not bad!)

Halle (Saale), 25. Februar 2010

List of publications

Hamasha HR, Hensen I (2009) Seed germination of four Jordanian *Stipa* spp: differences in temperature regimes and seed provenances. *Plant Species Biology*, **24**, 127–132.

Hamasha HR, Schmidt-Lebuhn AN, Schleuning M, Hensen I: Genetic diversity and population structure correlate with environmental factors in four *Stipa* species of Jordan, as revealed by AFLP markers. Submitted to *Ecography*.

Hamasha HR, Hagen KB, Röser M: *Stipa* (Poaceae) and allies in the Old World: Molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages. *Plant Systematics and Evolution* (Accepted pending minor revision).

Declaration of own contributions to the original articles

Because several co-authors contributed to the original articles in the following the percentage of own work is displayed.

Chapter 2: Genetic diversity and population structure correlate with environmental factors in four *Stipa* species of Jordan, as revealed by AFLP markers.

- Collection of plant materials and field data: 90%
- AFLP analysis: 100%
- Statistical analysis: 95%
- Manuscript: 90%

Chapter 3: Seed germination of four Jordanian *Stipa* spp: differences in temperature regimes and seed provenances.

- Collection of seeds and field data: 95%
- Germination test in the lab: 100%
- Statistical analysis: 100%
- Manuscript: 90%

Chapter 4: *Stipa* (Poaceae) and allies in the Old World: Molecular phylogenetics realigns genus circumscription and gives evidence on the origin of American and Australian lineages.

- Labratory work (DNA sequencing): 80%
- Data analysis, molecular phylogentics, statistical analysis: 80%
- Manuscript: 80%

Declaration of self-contained work / Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich diese Arbeit bisher weder der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zur Promotion vorgelegt habe.

Weiterhin erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegeben Hilfsmittel und Quellen verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht.

Im Übrigen erkläre ich, dass ich mich noch nie um einen Doktorgrad beworben habe.

Halle (Saale),

Hassan R. Hamasha