University of South Bohemia in České Budějovice

Faculty of Biological Sciences

Factors causing decline and extinction of the endangered species *Gentiana pneumonanthe* and *Maculinea alcon*

PhD. Thesis

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České Budějovice 2001

Dizertační práce / PhD. Thesis

Křenová Z. 2001: Factors causing decline and extinction of the endangered species *Gentiana pneumonanthe* and *Maculinea alcon*. PhD. Thesis, 99 pp., University of South Bohemia in České Budějovice, Faculty of Biological Sciences.

Školitel / Supervisor

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Anotace / Annotation:

Both field observations and manipulative experiments were carried out to evaluate critical phases of life cycles and define factors that effect the viability of populations of the rare plant species *Gentiana pneumonanthe* and its specialized herbivor *Maculinea alcon*.

Práce vznikla za podpory těchto grantů:

GA AV ČR 6141901
GA ČR 206/93/1179
GA ČR 206/96/0522
GA ČR 206/99/0889
Mattoni Awards 1998/1999
Mattoni Awards 2000/2001

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V Českých Budějovicích, 30. května 2001

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Chapter 1

Úvod / Introduction (in Czech)

Fragmentace stanovišť jako příčina vymírání druhů

V posledních desetiletích byl v České republice, obdobně jako i v ostatních evropských státech, zaznamenán výrazný úbytek druhů. Naprostá většina těchto druhů vyhynula v důsledku ztráty vhodných stanovišť. A v důsledku ničení vhodných stanovišť došlo u řady dalších druhů k radikálnímu snížení počtu populací a k výraznému zmenšení velikosti existujících populací.

U rostlinných druhů je možné za hlavní příčiny ničení stanovišť označit odvodňování, intenzifikaci zemědělství a lesnictví (přihnojování, pěstování monokultur, zavádění kulturních genotypů), znečištění prostředí, stavební aktivity (Pegtel 1998) a v případě lučních druhů, dále ještě ukončení hospodaření, s ním spojený nástup sukcese a zalesňování. Právě druhy lučních společenstev, která jsou v rámci Evropy hodnocena jako druhově nejbohatší společenstva (Klimeš 1991, Kull & Zobel 1991, Lepš 1999), patří mezi nejsilněji ohrožené a nejvíce ubývající. Ve střední Evropě, včetně Čech, je existence lučních společenstev, s výjimkou alpinských trávníků, maloplošných xerotermních porostů a travních společenstev v říčních nivách, podmíněna činností člověka. Mizení vhodných stanovišť, které s lidskými aktivitami přímo souvisí, probíhalo a stále ještě probíhá v různých evropských zemích různě intenzivně.

V Čechách byla výrazným mezníkem ve způsobech obhospodařování krajiny polovina 20. století. V období těsně po 2. světové válce bylo, v souvislosti s odsunem německého obyvatelstva, ukončeno extenzivní hospodaření v pohraničních oblastech, ve vnitrozemí se tento proces protáhl až do první poloviny 50. let. Na většině území postupně docházelo k intenzifikaci zemědělství, které v případě vlhkých luk znamenalo odvodnění a následný převod na ornou půdu nebo dosetí kulturní travní směsí. Většina ploch byla přehnojována organickými i anorganickými hnojivy. K odvodňování mokrých luk docházelo především na přelomu 50. – 60. let a dále v letech 70. Do těchto období je datován největší úbytek lokalit řady lučních druhů. Zbývající mokré louky v podhorských oblastech byly odvodněny v 1. polovině 80. letech v rámci tzv. náhradních rekultivací. Na většině neodvodněných luk se postupně v 50. a 60. letech 20. století přestalo hospodařit, protože ruční kosení nebo extenzivní pastva byly shledány jako málo produktivní. Louky byly ponechány sukcesnímu vývoji, některé byly zalesněny, často stanovištně nevhodnými monokulturami. Zalesňování pokračovalo i v 90. letech 20. století, kdy byla řada maloplošných lučních lokalit v restitučním řízení navrácena původním majitelům, kteří neměli zájem na nich hospodařit, a proto je, často nezákonně, zalesnili. Mezi jednotlivými regiony existují rozdíly v intenzitě i časovém období, kdy k výše popsaným změnám došlo. Obecně platí, že podhorské oblasti a oblasti na rozhraní okresů a krajů jsou zachovalejší.

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Zbytky druhově bohatých lučních porostů zůstaly na začátku 21. století zachovány především v maloplošných chráněných územích a v některých větších územích, které byly ušetřeny intenzifikace zemědělství. V případě maloplošných chráněných území (přírodní rezervace, přírodní památky) je třeba konstatovat, že teprve v posledních deseti až dvaceti letech došlo ke změně náhledu a v chráněných územích jsou prováděny asanační zásahy, které alespoň simulují původní způsob hospodaření (Petříček 1999). V minulosti převládající přístup, doporučující ponechat chráněná území bez zásahu člověka, uchránil některé lokality například před odvodněním či zalesněním, ale fakticky pouze oddálil jejich zánik. Navzdory prováděným zásahům je však životaschopnost populací řady druhů v chráněných územích silně ohrožena. Důvodem je především skutečnost, že rezervace jsou malé (většina lučních rezervací v ČR má výměru pouhých několik ha) a populace v nich přežívající jsou izolované od dalších populací stejného druhu. Odlišná je situace ve vojenských výcvikových prostorech, kde doposud existují životaschopné populace některých druhů z okolní krajiny téměř vymizelých. Ve většině případů se jedná o původně středně hustě osídlené oblasti (např. VVP Boletice cca 8500 obyvatel/219 km^2 v roce 1938 vs. 50 obyvatel v roce 2000; Pavlíčko 1997), které byly po 2. světové válce vysídleny. Kromě okrajových částí se v územích zemědělsky nehospodařilo. Meliorační zásahy nebyly prováděny téměř vůbec a vegetace na většině bezlesí byla ponechána samovolné sukcesi. Vojenská činnost byla omezena na malé části výměry (VVP Boletice – 82,2 km^2 z celkových 219 km^2) a některé vojenské aktivity (např. pojezdy těžkých vozidel, tanků, dopady střel nebo následné požáry) lokálně navracely vývoj vegetace do iniciálních stádií sukcese. Opomineme-li negativní aspekty působení vojska (např. kontaminace prostředí ropnými a chemickými látkami), jsou vojenské výcvikové prostory, bývalé i doposud používané, významnými územími z hlediska ochrany přírody nejen v České republice, ale i v ostatních evropských zemích (Valachovič 2000). Pro řadu druhů představují VVP poslední větší územní celky s výskytem vhodných stanovišť, která z okolní hustě obydlené středoevropské krajiny již vymizela.

Ničení vhodných stanovišť představuje fakticky několik procesů, které spolu souvisí. Jedná se samozřejmě o vlastní mizení lokalit, dále pak o fragmentaci zbývajících stanovišť a o snížení kvality zbývajících stanovišť (Hanski 1999). Fragmentací stanovišť je myšlena situace, kdy v původní oblasti výskytu se doposud zachovalá stanoviště nacházejí v menších a více či méně izolovaných skupinách ('patches' neboli fragmentech). Mizení lokalit a fragmentace se obvykle objevují současně, ale je třeba tyto dva procesy od sebe odlišit. V současné době, kdy postupně došlo k odklonu (Harrison & Bruna 1999; Hanski 1999 – obr. 10.1) od teorie ostrovní biogeografie (MacArthur & Wilson 1967) směrem k teorii metapopulací (Hanski 1996), a na krajinu je nahlíženo jako na síť, jejíž jednotlivé buňky buď jsou nebo nejsou vyplněny vhodným stanovištěm (Hanski 1999, sect. 5.1), je možné v počátečním období destrukce stanoviště, kdy jsou zbývající vhodná stanoviště stále ještě ve vzájemném kontaktu, považovat samotné přímé ničení stanovišť za významnější. V tomto období je předpokládaná velikost populace snížena na poměr, který odpovídá zbývající výměře stanoviště. Při pokračující destrukci stanovišť, když začne docházet k omezení kontaktu mezi fragmenty, je přímé ničení stanovišť posilováno i dalšími procesy spojenými s fragmentací. Mimo jiné se jedná o procesy snižování kvality stanoviště související s tzv. okrajovým efektem ('edge effect'; např. Primack 2000), tj. skutečností, že při fragmentaci stanoviště dochází k nárůstu okrajů vzhledem k výměře stanoviště (Yahner 1988).

S rostoucí fragmentací stanovišť se velikost populací snižuje více, než by odpovídalo výměře zbytkových stanovišť. Fragmentace stanovišť ovlivňuje přežívání druhů celou řadou faktorů, k nimž patří i vliv velikosti populace na produkci semen, jejich klíčivost a přežívání semenáčků (Morgan 1999, Kéry et al. 2000). Na malé izolované populace působí mnohem více okolní, pro druh nevhodné prostředí. Několik studií, většina na hmyzu, dokumentovalo vliv fragmentace stanovišť na druhovou bohatost, skladbu druhů a jejich abundanci (Didham et al. 1996, Hanski 1998, Steffan-Dewenter & Tscharntke 2000). Také u denních motýlů je ztráta a fragmentace stanovišť považována za hlavní důvod mizení mnohých druhů (Thomas et al. 1998, van Swaay& Warren 1999).

U rostlin je více pozornosti věnováno genetickým aspektům fragmentace populací a závěrům, které z toho vyplývají pro ochranu druhů. Základní teorie předpokládají, že zmenšení velikostí populací a zvýšení izolovanosti povede prostřednictvím zvýšeného působení genetického driftu, inbreedingu, sníženého genového toku mezi populacemi a zvýšené pravděpodobnosti extinkce subpopulací v metapopulaci k omezení genetické variability uvnitř populací a zvětšení mezipopulační genetické variability (van Treuren et al. 1991, Raijmann et al. 1994). Tyto skutečnosti přímo ovlivňují pravděpodobnost přežití druhu. Z krátkodobého hlediska může snížení heterozygozity snížit fittness jedinců a celkovou životaschopnost přežívající populace. Z dlouhodobého hlediska pak limituje schopnost druhu reagovat na selekční tlak (Frankel et al. 1995). U rostlin je vliv fragmentace ovlivněn sedentárním způsobem života, různou délkou života, překrýváním generací, kombinací sexuálního a asexuálního rozmnožování, přenosem genů ve formě pylu i semen a vztahem k opylovačům a vektorům rozšiřování.

Genetická variabilita populace, která je definovaná jako genetická variabilita všech jedinců v populaci a vyjadřuje se buď jako polymorfismus alel ('allelic richness'; A) nebo předpokládaná heterozygozita ('gene diversity'; He), může být v důsledku fragmentace stanovišť snížena dvěma způsoby. Za prvé přímo v době fragmentace dochází k efektu zúženého hrdla láhve ('genetic bottleneck'), protože přeživší individua nesou pouze omezený vzorek původních genotypů. Následně pak v malé izolované populaci pokračuje ztráta alel působením genetického driftu (Barret & Kohn 1991). Empirická data, mj. i na *Gentiana pneumonanthe* (Raijmann et al. 1994), potvrdila souvislost mezi fragmentací stanoviště a snížením genetické variability u populací se sníženou populační velikostí. Vliv působení genetického driftu je závislý na době, resp. počtu generací, po kterou je velikost populace malá.

Z tohoto důvodu je možné předpokládat, že druhy krátkověké budou mít vlivem genetického driftu sníženou genetickou variabilitu více než druhy dlouhověké (např. druhy r. *Gentianella* vs. *Gentiana*). Je také nutné zohlednit případné překrývání generací (Nunney 1993). Kromě genetické variability v rámci jednoho lokusu působí fragmentace stanovišť také na kvantitativní genetickou variabilitu, tedy fenotypovou variabilitu, která je pod kontrolou několika genů. Např. Ouberg et al. (1991) zaznamenali pozitivní korelaci mezi velikostí zbytkové populace *Scabiosa columbaria* a *Salvia pratensis* a jejich růstem. Také Fischer et al. (2000) zjistili u klonálního druhu *Ranunculus reptans* vztah mezi velikostí malých populací a jejich fittness ('Allee effect'). Naopak u *Gentiana pneumonanthe* zaznamenali

Oostermeijer et al. (1994a) mizivý vztah mezi velikostí populace a několika růstovými charakteristikami. Výsledky studií týkající se kvantitativní variability jsou mnohem méně jednoznačné a hůře interpretovatelné než výsledky studií allozymové variability (Young et al. 1996).

Genetická variabilita na úrovni jedince je popisována mírou heterozygozity (= podílem heterozygotních alel v genotypu jedince; H), která je ovlivněna frekvencí alel na lokusu, inbreedingem, typem a intenzitou selekce. Fragmentace stanovišť snižuje heterozygozitu dvěma způsoby. Pokles heterozygozity je přímým důsledkem snížené diverzity genů ve zbytkové populaci, ale významnější je snížení v důsledku inbreedingu v rámci zbytkové populace. K inbreedingu dochází při samooplození nebo při oplození mezi geneticky úzce příbuznými jedinci. Nezanedbatelnou roli hraje také vliv fragmentace stanoviště na chování opylovačů (Kearns et al. 1998). Inbreeding se začne projevovat okamžitě po fragmentaci stanoviště, a proto se ve zbytkové populaci nejdříve projeví snížení heterozygozity, za nímž následuje snížení alelického polymorfismu a genové diverzity. Vliv velikosti populace na podíl heterozygotů v populaci byl potvrzen i pro *Gentiana pneumonanthe* (Raijmann et al. 1994). Ztráta variability v populacích byla vyšší než by odpovídalo pouze vlivu „úzkého hrdla láhve“, tedy bottlenecku, a genetického driftu, a proto byla interpretována jako působení inbreedingu. Naopak při studiu malých populací *Salvia pratensis* a *Scabiosa columbaria* (Ouberg et al. 1991) nebyl vliv inbreedingu potvrzen.

S ubýváním heterozygotů v populaci bývá spojováno snížení fittness, které vede ke snížení životaschopnosti populace. Tento děj, nazývaný ′inbreeding depression′, je vysvětlován na základě dvou různých modelů (Charlesworth & Charlesworth 1987). Tzv. ′partial′ nebo-li ′complete model′ předpokládá snížení fittness z důvodu zvýšení podílu homozygotů nesoucích škodlivé alely. ′Overdominant model′ předpokládá, že heterozygotní jedinci mají vyšší fittness, a v důsledku ubývání heterozygotů v populaci se snižuje celková vitalita populace. V současnosti je upřednostňován ′partial model′. Konktrétních příkladů, které by dokumentovaly přímou souvislost mezi fragmentací stanoviště a snížením heterozygozity a fittness populace není příliš mnoho, ale jedním z nich je výsledek studie na *Gentiana pneumonanthe* (Oostermeijer et al. 1994a), ve které byl potvrzen pozitivní vztah mezi mírou heterozygozity malých populací a jejich fittness. S ′inbreeding depression′ souvisí také úvahy o cílené genetické zátěži (′purging genetic load′), v důsledku které by inbreeding v malých populacích společně se selekcí proti homozygotům s nízkou fittness, vedl k eliminaci škodlivých alel z populace a následnému zvýšení fittness populace (Barrett & Charlesworth 1991). Výsledky několika studií tuto teorii u rostlin nepotvrdily (van Treuren et al. 1993, Widen 1993).

Jak již bylo uvedeno, jedním z předpokládaných důsledků fragmentace stanovišť je snížení mezipopulační výměny genů. Tato skutečnost platí především pro druhy, jejichž pyl a semena jsou rozšiřována větrem, protože vzdálenost mezi populacemi přímo ovlivňuje tok genů. U druhů, pro něž je vektorem některý druh živočichů, je vliv fragmentace nepřímý. Jak již bylo uvedeno, u malých izolovaných populací stoupá mezipopulační variabilita vlivem genetického driftu, ale již minimální migrace mezi populacemi (odhaduje se jeden migrant za generaci) dokáže zamezit ztrátě alel vlivem genetického driftu (Wright 1931). Také v případě metapopulací může fragmentace stanovišť v důsledku ovlivnění frekvencí kolonizací a extinkcí nebo změnou počtu kolonistů zvýšit genetickou variabilitu mezi zbytkovými populacemi (Wade & McCauley 1988). Teorie předpokládá, že pokud extinkce a kolonizace převýší počet migrantů dvakrát, pak dojde k výraznému snížení genetické variability mezi subpopulacemi v rámci metapopulace (Lande 1992, Harrison & Hastings 1996, Hanski 1999).Také časové a prostorové rozrůznění migrací ovlivňuje variabilitu mezi subpopulacemi (Whitlock 1992), vliv času je významnější.

Poslední zde zmíněnou teorii související s fragmentací stanoviště je 'outbreeding depression' (Barret & Kohn 1991). Je to proces, v rámci kterého může dojít při promíchání dvou skupin genotypů ke snížení fittness populace. Tato situace může nastat především v případech, kdy se jedná o narušení genetické struktury populace adaptované na specifické lokální podmínky. Fischer & Matthies (1997) zaznamenali působení 'outbreeding depression' u *Gentianella germanica*. Některé další studie existenci 'outbreeding depression' potvrzují, jiné ji popírají, ale přesto je třeba provádět umělé křížení jedinců z malých navzájem izolovaných populací velmi obezřetně (Schierup & Christiansen 1996, Frankham 1999, Fenster & Galloway 2000).

Vliv fragmentace stanovišť na přežívání druhů je možné shrnout jako proces, v rámci kterého je výrazně ovlivněna populační genetika malých zbytkových populací. Obvykle dochází ke snížení genetické variability, vyjádřené buď počtem polymorfních alel, nebo heterozy~~r~~g ozitou jedinců. Snížení genetické variability je způsobeno efektem zúženého hrdal láhve ('bottleneck') a následným inbreedingem v malých populacích. Třetí v řadě je vliv genetického driftu.

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Není náhodou, že řada populačně genetických aspektů fragmentace stanovišť byla dokumentována na studiích prováděných na druhu *Gentiana pneumonanthe*. Tento druh, společně s celou řadou dalších druhů čeledi *Gentianaceae*, patří mezi druhy v současnosti vzácné a silně ohrožené, které však byly původně alespoň lokálně časté. Pro tyto tzv. nově vzácné druhy ('recently rare'), které se liší od vždy vzácných druhů ('naturally rare'), představuje fragmentace stanovišť a izolace lokalit vážné ohrožení životaschopnosti populací (Huenneke 1991). U druhů vždy vzácných (endemitů, druhů s malým nebo ostrůvkovitým areálem) se vyvinuly adaptace zachovávající životaschopnost populací i za těchto podmínek (Karron 1987, 1991), které však nově vzácným druhům chybí.

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S ohledem na tyto skutečnosti je u nově vzácných druhů nezbytné rozlišit kritické fáze vývojových cyklů, určit podmínky umožňující zachování životaschopnosti stávajících populací a rozpoznat faktory podmiňující úspěšnou reprodukci druhů. Důvodem je nejenom ochranářská snaha o zachování životaschopnosti populací, ale především možnost poznat a pochopit celou řadu evolučních a ekologických procesů.

Úroveň prozkoumanosti populační biologie taxonů rodu *Gentiana*, *Gentianella* a *Gentianopsis* (*Gentianaceae*) v Evropě

[illegible]

Mezi taxony, jejichž početnost i velikost populací se v posledních desetiletích rapidně snížila téměř ve všech evropských zemích, patří kromě často zmiňovaných vstavačovitých (*Orchideaceae*), také druhy čeledi *Gentianaceae*. Většina těchto druhů je v územích svého výskytu zařazena mezi druhy chráněné a je uvedena v Červených seznamech příslušných států. S ohledem na nutnost rozlišit kritické fáze vývojového cyklu druhů, určit podmínky umožňující zachování životaschopnosti stávajících populací a najít faktory podmiňující úspěšnou reprodukci druhu, byly v řadě zemí Evropy zpracovány studie zaměřené na jednotlivé aspekty populační biologie druhů. Výzkum stále ještě pokračuje a v současné době se připravuje koordinační projekt (European Research Network on Gentianaceae), jehož cílem je diskutovat a následně shrnout poznatky z jednotlivých zemích. Zachování životaschopných populací hořcovitých je důležité také proto, že na jejich existenci je vázána řada hmyzích druhů. V některých případech se jedná i o monofágy, jejichž úspěšný vývoj je podmíněn existencí dostatečně velké populace hostitelské rostliny. Příkladem mohou být vzácní modrásci z rodu *Maculinea*, jejichž housenky se v počátečních fázích vývojového cyklu živí na *Gentiana cruciata* a *Gentiana pneumonanthe* (*Maculinea rebeli, M. alcon*), příležitostně i na jiných druzích rodů *Gentiana* a *Gentianella*.
Biologie druhu *Maculinea alcon* je podrobně popsána v kapitole 6.

Reprodukční biologie

V 90. letech 20. století bylo v různých částech Evropy uskutečněno několik studií hledajících odpovědi na otázky týkající se regenerace druhů rodů *Gentiana* a *Gentianella*. U hořečků byla studována reprodukční biologie *Gentianella campestris* (Milberg 1994), *G. germanica* (Fischer & Matthies 1998b, Luijten et al. 1998, 1999), *G. bohemica* na populacích v Bavorsku (Rösler 1996) a *G. campestris* (Lennartsson et al. 1997). V Českých zemích byla sledována klíčivost u několika druhů hořečků: *Gentianella baltica* a *G. germanica* agg. (Zahradníková 1995), *Gentianella bohemica* (Reitschläger – nepublikováno). Několik autorů se věnovalo také ~~otázkám týkajícím se~~ opylování hořečků: *Gentianella amarella, G. campestris* (Lennartsson 1997) a *G. uliginosa* (Petanidou et al. 1998). V současnosti se reprodukční biologií několika druhů rodu *Gentianella* zabývá J. Brabec, PřF UK Praha.
Z druhů rodu *Gentiana* byla zkoumána reprodukční biologie u *Gentiana pneumonanthe* (Křenová & Lepš 1996, viz kapitola 2) a *G. lutea* (Kéry et al. 2000). Pokusy na druzích *Gentiana pannonica, G. cruciata* a *Gentianopsis ciliata* dosud probíhají. Opylování bylo zkoumáno u *Gentiana cruciata* (Petanidou et al. 1995a) a *G. pneumonanthe* (Petanidou et al. 1995b, 2001).

populac.

Demografie

Několik demografických studií se snažilo zachytit vývoj populací v různých typech vegetace. Byla studována demografie populací *Gentianella germanica* (Fischer & Matthies 1998b) a *G. campestris* (Lennartsson – nepublikováno).Také v České republice bylo uskutečněno několik menších studií. Reitschläger (nepublikováno) studoval vliv okolní vegetace na populační strukturu populací *Gentianella bohemica* v jižních Čechách. Pro několik lokalit existují alespoň informace o struktuře vegetace a případně o půdních podmínkách: *Gentianella bohemica* – 1 lok. (Neuhäusl & Neuhäuslová 1989), *G. baltica* – 1 lok. (Kolbek et al. 1994), *G. baltica* – 1 lok. a *G. bohemica* agg. – 2 lok. (Zahradníková 1995). Několik dalších demografických sledování druhů rodu *Gentianella* dosud pokračuje (koordinuje J. Brabec, PřF UK Praha).

Z druhů rodu *Gentiana* byla sledována demografie pouze u *G. pneumonanthe* (Chapman & Rose 1982, Rose et al. 1998, Oostermeijer et al. 1994b, 1995c, 1996). Výsledky těchto studií jsou zmíněny v následující podkapitole. V Čechách nebyly demografické studie druhů *Gentiana pannonica, G. cruciata* a *Gentianopsis ciliata* ještě ukončeny.

Populační genetika

Důležité informace přinesly studie populační genetiky. Populační genetika *Gentianella germanica* byla zkoumána na populacích ve Švýcarsku (Fischer & Matthies 1997, 1998a,c) a genetická diverzita byla sledována v populacích *Gentianella austriaca* ve východním Rakousku (Greimler & Dobes 2000). Řada studií se zabývala populační genetikou *Gentiana pneumonanthe* (Oostermeijer et al. 1994a, 1995a,b,c, 1998, Raijmann et al. 1994). Dosud pouze předběžné výsledky přinesly probíhající studie populační genetiky *Gentiana pannonica, G. pneumonanthe, G. cruciata* a *Gentianella bohemica* prováděné na českých populacích (Čurn a Křenová).

Jak již bylo uvedeno, řada pokusů a sledování jednotlivých druhů uskutečňovaných na území České republiky dosud pokračuje. Převážná část těchto experimentů je součástí tříletého projektu, financovaného Grantovou agenturou AV ČR (32-6141901), který je zaměřen na studium populačně biologických a genetických vlastností vybraných druhů z čeledi *Gentinaceae* a ekologických nároků, které jsou podmínkou pro přežití těchto druhů. Výsledky pokusů a sledování týkajících se druhu *Gentiana pneumonanthe*, které jsou prezentovány v následujících kapitolách této práce jako jednotlivé články, jsou součástí tohoto projektu.

Základní údaje z biologie druhu *Gentiana pneumonanthe*

Gentiana pneumonanthe, hořec hořepník, je vytrvalá bylina, patřící do sekce Pneumonanthe. Většina druhů sekce se vyskytuje v Severní Americe a východní Asii, z evropských druhů patří do této sekce *Gentiana asclepiadea*. Dospělé rostliny vytvářejí (15-) 20 – 40 (-60) cm vysoké většinou nevětvené lodyhy, přízemní listová růžice chybí. Spodní listy jsou malé, často šupinaté, horní kopinaté, vzácněji vejčité, na špici tupé, často podvinuté. Jednotlivé lodyhy nesou 1 - 25, zřídka více, modrých nálevkovitých květů. Ve vřetenovitém semeníku dozrává 300 – 700 (1000) velmi drobných semen. Podrobný morfologický popis druhu uvádějí Kirschner & Kirschnerová (2000). U mladých rostlin se 2-4 (-8) páry listů je primární kořen nahrazen kořeny adventivními obvykle již v průběhu prvního (max. druhého) roku života. Adventivní kořeny vyrůstají vodorovně z nejvyšší části mesokormu (viz fotografie v příloze). Na podzim se na bázi rostliny objevuji tzv. přezimující lodyhy ('overwintering shoots'), což jsou pouze několik cm vysoké výhony porostlé šupinatými listy. Z těchto přezimujících orgánů vyrůstají v následujícím roce nové lodyhy. Morfologická plasticita tohoto druhu je velmi široká (viz fotografie v příloze) a vedla k popsání různých podruhů a variet (Simmonds 1946, Hegi 1975), jejichž taxonomická hodnota je diskutabilní.

Gentiana pneumonanthe se vyskytuje v temperátní zóně Evropy a Asie. V Evropě se objevuje v oblastech s nižší nadmořskou výškou (do 1200 m n.m., Hegi 1975). Ve střední Evropě roste na nehnojených vlhkých loukách a extenzivních pastvinách, často na stanovištích s kolísající podzemní vodou. Půdní pH se pohybuje mezi neutrálními až mírně alkalickými hodnotami. V západní Evropě roste hořepník na vlhkých kyselých vřesovištích, okrajích rašelinišť a vlhkých loukách (Simmonds 1946, Chapman et al. 1989, Oostermeijer 1992, 1994b, 1998, Kesel & Urban 1999). V České republice, resp. v jihozápadních Čechách, kde byl výzkum převážně prováděn, se *G. pneumonanthe* vyskytuje v různě sukcesně vyvinutých společenstvech svazu Molinion a Violion-caninae. Některé zbytkové populace přežívají v okrajích borových nebo olšových lesů.

Z abiotických faktorů působí na biologii *Gentiana pneumonanthe* především zaplavení a oheň (Chapman et al. 1989), ale oba tyto faktory hrají mnohem větší roli na západoevropských vřesovištích. Hořepník snáší zaplavení velmi dobře a krátkodobé záplavy mají pozitivní vliv na generativní rozmnožování druhu (Kesel & Urban 1999). Některé české lokality jsou nepravidelně zaplavované. V Čechách nebyly hořepníkové lokality, na rozdíl od Anglie, nikdy pravidelně vypalovány.

Gentiana pneumonanthe je dlouhověký hemikryptofyt, dospělé rostliny mohou žít i několik dekád (Oostermeijer et al. 1994b, Rose et al. 1998). Přesný věk není možné u tohoto druhu určit nedestrukčními postupy. Na základě demografických studií bylo popsáno šest ontogenetických stádií: semenáčky, mladé (juvenilní) rostliny, sterilní dospělé rostliny, plodné dospělé rostliny, dormantní jedinci (Oostermeijer et al. 1994b). Dostupná data o biologii druhu v bezkolencových loukách umožnila sestavení diagramu životního cyklu s odhady pravděpodobnosti přechodů mezi jednotlivými stádii (obr. 3, kapitola 2). Věk jedinců je možné odhadnout na základě stavby podzemních orgánů. Každým rokem vyrůstají horizontálně z nejvyšší části mesokormu nové adventivní kořeny, které tak vytvářejí jakási patra, která odpovídají stáří jedinců (viz fotografie v příloze). Tímto způsobem růstu je báze rostliny stále udržována na povrchu půdy i na stanovištích, kde každoročně dochází k velké akumulaci odumřelé biomasy.

Fenologicky patří *Gentiana pneumonanthe* ke druhům pozdnějším. Semena klíčí od konce března do června. První lodyhy se obvykle objevují počátkem května. Hořepníky kvetou od července do konce srpna. Semena dozrávají v září až říjnu a od konce srpna se již začínají vytvářet přezimující lodyhy. V jednotlivých letech může v důsledku klimatických výkyvů dojít k posunu fenologických období. Mezi populacemi existují značné fenologické rozdíly, často způsobené adaptacemi rostlin na pravidelný termín obhospodařování lokality.

Gentiana pneumonanthe se na rozdíl od některých jiných hořců rozmnožuje pouze semeny. Protoandrické květy opylují především čmeláci, velmi drobná bezkřídlá semena jsou ze semeníku roznášena mechanicky a částečně větrem pouze na malé vzdálenosti. *G. pneumonanthe* patří mezi ty druhy rostlin, které k úspěšnému vyklíčení semen a uchycení semenáčků potřebují tzv. bezpečná místa ('safe sites'). Pro hořepníky jsou takovými místy plošky s obnaženým půdním povrchem ('gaps'; podrobnosti viz kapitoly 3 a 4). Zatímco pro klíčení semenáčků je nezbytné narušení zapojeného drnu, dospělé rostliny mohou řadu let přežívat na neobhospodařovaných lokalitách s hustou vegetací a velkou vrstvu opadu. Především z dlouhodobého hlediska je vitalita těchto populací omezená, často dochází k omezení cizosprášení, protože jedinci jsou izolováni okolní vegetací a opylovači upřednostňují opylování květů v rámci jedince před náročným hledáním dalších rostlin. Pokud jsou hořepníky zastíněny okolní vegetací příliš intenzívně a dlouho, jsou lodyhy polehlé, květů je jen několik, řada z nich není vůbec opylena a většina semeníků je zničena plísní nebo herbivory. Podle zastoupení jednotlivých ontogenických fází v populacích hořepníku byly vylišeny tři základní typy populací (Oostermeijer et al. 1994b). Je-li převažující zastoupení semenáčků a juvenilních rostlin, označuje se populace jako 'invazní'. Ve 'stabilní' populaci jsou všechna ontogenická stádia zastoupena rovnoměrně a u 'senilních' populací chybí semenáčky a juvenilní rostliny, v populaci přežívají pouze dospělci. Přehled stavu studovaných populací *G. pneumonanthe* v oblasti jihozápadních Čech je uveden v obr. 6, kapitola 2.

Na řadě lokalit je část plodných rostlin *Gentiana pneumonanthe* každoročně poničena okusem spárkatou zvěří. Také dobytek hořepníky vyhledává pro jejich atypickou hořkou chuť, a pokud se na pastvinách vyskytuje pouze pár kvetoucích jedinců, bývají často okousány zcela. Okus v jarních měsících stimuluje vytváření nových výhonů z adventivních pupenů, a vede ke zvýšení počtu květů na rostlině, později v létě rostliny již neregenerují. Produkce semen z náhradních květů se blíží nule. Především semenáčky a mladé rostliny bývají silně poznamenány okusem slimáků a drobných hlodavců. Také mezi hmyzem je řada herbivorů *G. pneumonanthe*, z nichž nejzajímavější je kriticky ohrožený, monofágní modrásek *Maculinea alcon* (viz kapitola 6).

Populačně genetické studie prováděné na *Gentiana pneumonanthe* v západní Evropě (Raijmann et al. 1994, Oostermeijer et al. 1995a, b, c) mimo jiné v souladu s teoretickými předpoklady potvrdily, že v malých populacích tohoto druhu dochází ke snížení genetické variability a zastoupení heterozygotů v populacích. Populačně genetické studie na českých populacích dosud pokračují, ale předběžné výsledky naznačují, že snížení heterozygozity a polymorfismu v českých populacích není tak výrazné jako v západní Evropě (tabulka 3, kapitola 2).

Závěrem je možné shrnout, že *Gentiana pneumonanthe* je typickým představitelem vzácných druhů, které byly ještě v 60. letech 20. století dosti hojné. Například ze 16 lokalit uváděných Vaněčkem (1969) na Horažďovicku existují v současnosti 2, ze 4 lokalit na Plzeňsku (Hadač et al. 1968) vymírá poslední. V rámci celých jižních Čech je druh hodnocen jako dříve dosti hojný, dnes už je vzácný a jen někde ještě roztroušený (Chán 1999). V regionu JZ (konkrétně okresech CH, KV, SO, TC, PM, PS, PJ, DO, KT, RO, RA, BE, ST, PC, TA – jih, CB, CK) bylo v letech 1994 – 2000 zaznamenáno 52 recentních populací *Gentiana pneumonanthe*. Jejich populační velikost kolísala od jednoho do několika set jedinců a životaschopnost populací byla různá, v průběhu studie několik populací vyhynulo (podrobnější údaje viz appendix 1 v kapitole 2).

České abstrakty kapitol 2 - 6

II. Biological flora of Central Europe: *Gentiana pneumonanthe* L.

Biologická flóra Střední Evropy: *Gentiana pneumonanthe* L.

Gentiana pneumonanthe je dlouhověká trvalka vyskytující se na živinami chudých vlhkých loukách a kyselých vřesovištích. Tento původní druh středoevropské květeny patří v současnosti mezi druhy ohrožené. Článek přináší přehled o morfologii, rozšíření, ekologii, populační biologii a genetice tohoto druhu. *Pozn.: Nejdůležitější fakta jsou uvedena v předcházející kapitole.*

III. Regeneration of a *Gentiana pneumonanthe* population in an oligotrophic wet meadow

Obnova populace *Gentiana pneumonanthe* ve vlhké oligotrofní louce

V rámci terénního experimentu byl testován vliv různých podmínek na klíčení a uchycení semenáčků a dále byl zkoumán kompetiční vliv okolní vegetace na dospělé jedince *Gentiana pneumonanthe.* Semena hořepníku byla vyseta do různě obhospodařovaných ploch (pokosená, vypálená louka, louka s narušeným zapojením drnu a nepokosená louka jako kontrola) uspořádaných jako úplně znáhodněné bloky. Statisticky nejvyšší klíčivost byla zaznamenána na plochách s narušeným drnem, nejméně semen vyklíčilo na plochách kontrolních. Také v přežívání vyklíčených semenáčků byly zaznamenány rozdíly mezi jednotlivými typy zásahů. Po dvou letech přežívaly semenáčky pouze v plochách s narušeným drnem. Vliv okolní vegetace na dospělé rostliny byl testován v pokusu, kde vegetace v okolí poloviny označených rostlin byla v průběhu vegetační sezóny pravidelně ostříhávána, zatímco druhá polovina rostlin byla ponechána v zapojeném porostu. Žádné statisticky průkazné rozdíly ve vitalitě hořců z různých skupin nebyly zjištěny. Na základě těchto pokusů bylo konstatováno, že fáze vyklíčení a uchycení mladých rostlin se jeví jako kritická pro zachování populace.

IV. Survival and viability of *Gentiana pneumonanthe* in differently managed plots over four years

Přežívání a vitalita mladých jedinců *Gentiana pneumonanthe* v různě obhospodařovaných plochách, čtyřletý experiment

V průběhu čtyřletého experimentu bylo zkoumáno uchycení semenáčků a přežívání a vitalita mladých jedinců *Gentiana pneumonanthe.* Ve skleníku předpěstované semenáčky byly vysazeny do gapů o průměru 5, 10, 20 cm a do ploch bez gapů. Gapy byla umístěny v kosených a nekosených plochách vlhké oligotrofní louky, na které bylo hospodaření ukončeno 14 let před zahájením pokusu. Vitalita mladých hořců byla charakterizována několika parametry (IGVs): počtem listů, lodyhů, květů a výškou rostlin. Mortalita rostlin byla dosti vysoká. V průběhu první zimy uhynulo více jak 50% jedinců, ale ze všech přeživších rostlin jich 25% ve čtvrtém roce vykvetlo. Množství uhynulých jedinců v různě velkých gapech a různě obhospodařovaných plochách se měnilo v jednotlivých letech. Kosení a velikost gapů ovlivnilo pouze některé IGVs. Výsledky RDA ukazují rozdíly ve vegetační skladbě ploch ve kterých hořce přežily a uhynuly. V plochách s nejvyšší mortalitou hořců byl zaznamenán zvýšený výskyt širokolistých druhů trav a druhů *Prunella vulgaris* a *Pedicularis sylvatica*, které často zcela pokryly uměle vytvořené gapy. Studie potvrdila nezbytnost gapů pro regeneraci *G. pneumonanthe*. Přestože byla ve velkých gapech zaznamenána na začátku experimentu větší mortalita způsobená extrémnějšími výkyvy mikroklimatických podmínek, jsou větší gapy nezbytné v dlouhodobě neobhospodařovaných loukách. V pravidelně obhospodařovaných loukách postačí existence přirozeně vznikajících malých gapů.

V. Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L.

Vztahy mezi reprodukčním úspěchem vzácného druhu *Gentiana pneumonanthe* L. a řadou jeho populačních a stanovištích charakteristik.

Na 17 nizozemských populacích druhu *Gentiana pneumonanthe* byly testovány závislosti mezi charakteristikami prostředí i populací a produkcí vajíček, semen a seed-set. Cílem této studie bylo odhalit význam vlivu velikosti populace a genetické variability populace na produkci semen, pokud jsou zohledněny i další potencionálně významné faktory prostředí. Bylo zjištěno, že na lokalitách s nižší půdní vlhkostí a vyšší koncentrací živin je vyšší pokryvnost trav a méně vřesovištních druhů. Na takových stanovištích jsou populace *G. pneumonanthe* malé a heteryzygozita potomků je nízká. Počet vajíček v semenících byl negativně korelován s heterozygozitou, fosforem v půdě a pH. Pozitivní korelace byla mezi počtem vajíček a obsahem vápníku, draslíku, SO_4, NH_4 a konduktivitou. Seed-set a počet životaschopných semen byl pozitivně korelován s velikostí populace a přítomností rostlin kvetoucích ve stejnou dobu jako hořce. Váha semen byla nezávislá na všech měřených faktorech. Semeníky rostlin z malých a velkých populací se nelišily v počtu vajíček, ale lišily se v reprodukční úspěšnosti. Genetická variabilita populací se neukázala být příliš významnou. Kromě velikosti populace se jako velmi významná ukázala přítomnost druhů kvetoucích ve stejnou dobu a obsah některých živin v půdě.

VI. Factors causing decline and extinction of the Blue Alcon butterfly in the SW of the Czech Republic, Central Europe

Faktory způsobující úbytek a vyhynutí modráska hořcového (*Maculinea alcon*) v jihozápadní části České republiky

Více než 50 lokalit *Gentiana pneumonanthe*, hostitelské rostliny monofága *Maculinea alcon*, bylo detailně zkoumáno v letech 1996-2000 v JZ části Čech. Celkem 18 populací *M. alcon* bylo objeveno v oblasti, kde před rokem 1995 byla známa pouze jedna lokalita. Na sledovaných lokalitách byla zaznamenána řada ekologických charakteristik s cílem odhalit klíčové faktory limitující výskyt modráska. Nebyly zjištěny statisticky významné rozdíly ve vegetační skladbě lokalit s a bez výskytu modráska. Jako nejlepší prediktor výskytu motýla se ukázala být velikost populace hostitelské rostliny (p<0,001). Na zkoumaných lokalitách byla zjištěna přítomnost všech tří potencionálních mravenčích hostitelů z rodu *Myrmica* (*Myrmica rubra, M. ruginodis*, and *M. scabrinodis*), housenky modráska byly nalezeny v hnízdě nejběžnějšího z nich – *M. scabrinodis*. Z klimatických dat se jako dobré prediktory přítomnosti modrásků na lokalitě ukázaly být průměrný roční úhrn srážek a počet deštivých dnů v létě. V roce 1996 bylo v jižní části zkoumané oblasti objeveno několik nových populací *M. alcon*. V roce 1997 tyto populace vyhynuly. Při porovnání řady biotických a abiotických charakteristik lokalit z doby před a po vyhynutí nebyly zjištěny žádné významné rozdíly. Dlouhodobá klimatická data ukázala, že průměrné roční srážky a počet dnů se sněhovou pokrývkou je vyšší a průměrné roční teploty nižší v této oblasti v porovnání s ostatními oblastmi výskytu *M. alco*n v JZ Čechách. Podrobná data z let 1994 – 99 zachytila výrazné klimatické výkyvy v letech 1996 a 1997. Na základě zjištěných skutečností bylo konstatováno, že atypický vývoj počasí v letech 1996 a 1997 způsobil vyhynutí druhu na okraji jeho klimatického areálu.

Poděkování

Především bych chtěla poděkovat svému školiteli a kamarádovi Janu Š. Lepšovi, bez jehož odborného vedení a nekonečné trpělivosti, by tato práce nikdy nevznikla, a já bych s velkou pravděpodobností dělala něco úplně jiného.

Děkuji své rodině i mnohým přátelům, kteří mě podporovali ve chvílích slabosti. Zvláštní poděkování patří mé mamince a Sojkovi, kteří se trpělivě a bez nároku na honorář, stávali mými technickými pomocníky, často i při činnostech, které jim připadaly jen málo smysluplné.

Velmi děkuji Milanu Štechovi, Slávce Nesvadbové, Jaromíru Sofronovi, Miroslavě Šandové, Ivoně Matějkové, Lence Pivoňkové, Václavu Chánovi, Františku Procházkovi, Oldřichu Buškovi, Petru Mudrovi, Jirkovi Hadincovi, Aleně Vydrové, Láďovi Melicharovi a mnohým dalším, bez jejichž znalostí lokální flóry, by tato práce nemohla nikdy vzniknout.

Lojzovi Pavlíčkovi děkuji za mnohem víc, než jen za to, že mi ukázal krásy Boleticka.

Za odborné konzultace a mnohé podnětné diskuse děkuji Gerardu Oostermeijerovi, Hans den Nijsovi, Janu Štěpánkovi, Ludmile a Janu Kirschnerovým, Františkovi Krahulci, Ladislavu Čurnovi, Jirkovi Brabcovi, Marii a Petrovi Šmilauerovým, Martinu Konvičkovi, Rudi Mattonimu, Karlu Špitzrovi, Josefu Jarošovi a mnohým dalším.

Johnovi a Priscille Tithus děkuji za nekonečnou trpělivost s jakou opravovali stále stejné chyby v mých anglických textech.

Mé poděkování patří také kolegům z Agentury ochrany přírody a krajiny ČR, kteří mi pomáhali ve snahách o záchranu některých hořcových a modráskových lokalit.

V neposlední řadě děkuji všem, kteří mohou za to, že Biologická fakulta byla taková, jaká byla.

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Chapter 2

Biological flora of Central Europe: *Gentiana pneumonanthe* L.

Biological flora of Central Europe: *Gentiana pneumonanthe* L.

Zdeňka Křenová

Will be submitted to Flora

Summary

Gentiana pneumonanthe is a long-aged perennial herb of nutrient poor wet meadows and acid heathlands. It is native to Europe, where it has become an endangered species. This article reviews morphology and taxonomic status, and distribution, ecology, population biology and genetics of this taxon with stress on situation in Central Europe.

Key words: *Gentiana pneumonanthe*, ecology, species biology, Central Europe, endangered species.

1. Taxonomy and morphology

1.1. Taxonomy

Gentiana pneumonanthe L. (Gentianaceae) - marsh gentian, Lungen-Enzian
Section: *Pneumonanthe*
Synonyms:
Pneumonanthe vulgaris F. W. SCHMIDT Fl. Boëm. Inch. 2: 14,1794
Ciminalis pneumonanthe (L.) BORKH. Arch. Bot. 1:26, 1796
Gentianusa pneumonanthe (L.) POHL Tent. Fl. Bohem. 1:167, 248,1809
Dasystephana pneumonanthe (L.) SOJÁK Čas. Nár. Muz. 148:200, 1980
Tretorhiza pneumonanthe (L.) Á. et D. LÖVE Taxon 35: 899, 1986

1.2. Morphology

Gentiana pneumonanthe is a long-lived, polycarpic perennial herb. Reproductive plants without basal leaf rosettes have 1-10 (-15) glabrous stems occasionally with a ligneous base. Flowering stems are usually erect and unbranched, (15-) 20-40 (-60) cm high. Branches may appear in later flowering stages (SZUJKÓ-LACZA & SEN 1977). Tiny branches grow from adventitious buds located in nodes after the top of the plant has been cut. In comparison to several other *Gentiana* species *(G. asclepiadea, G. cruciata)*, which have a normal distribution of internode lengths, *G. pneumonanthe* shows an irregular pattern in internode length (SZUJKÓ-LACZA & SEN 1977). Shaded plants have flattened stems. Lower

[illegible]

[illegible]

leaves are small and often scaly. Upper leaves are slightly revolute with blunt tops. They are linear or occasionally lanceolate (2.5-) 3.0-4.5 (-5.0) cm long and 0.2-0.5 (-1.2) cm wide, often longer than internodes. Leaves of adult plants in full sun are bright or dark green, while leaves of shaded plants are thin, light green or yellow-green.

Stems usually bear 1 - 25 protandrous flowers. Flowers are terminal, or terminal and axillary, or subsessile. The calyx has 5 linear-lanceolate teeth. The corolla is 25-50 mm long and deep blue (rarely rose or white) with 5 greenish stripes. There are usually 5 corolla lobes but atypical plants with 4 or 6 lobes occur. Flowers have 5 stamens and coherent anthers. From 300 to 700 (1000) seeds ripen in a capsule. Seeds are very small (0.25-0.35 x 1.50-1.85 mm), unwinged, light (0.32 mg on average) and contain very little endosperm (SALISBURY 1942, PETANIDOU 1991, PETANIDOU ET AL. 1995).

Juvenile plants produce a rosette with 2-4-(8) leaves. Adventitious roots replace the primary root very early (KUTSCHERA & LICHTENEGGER 1992), usually during the first (second) year of individual life. New adventitious roots grow up horizontally from the highest part of a mesocorm (caudex) that is created from condensed internodes *. KARRER (1996) described a similar structure of underground organs for *Gentiana asclepiadea*, the relative species of *G. pneumonanthe*. Usually several (2-5) over-wintering shoots (2-4-(6) cm) with only scale-like leaves appear in late summer. The following spring new stems grow from the over-wintering shoot but the number of stems per individual is usually higher than the number of over-wintering shoots from the previous autumn.

rhizom ?

Morphological plasticity of this species is high. Abiotic conditions, land management, and other factors can influence morphological plasticity. Plants differ in size, leaf size and shape, habit, number of shoots, and flower number and colour. Many forms and varieties have been described in accordance with these morpho-types but the taxonomic value of these distinctions are unclear. HEGI (1975) distinguished the following forms and varieties: f. *latifolia* Schollet (unbranched stems, linear leaves, only one top flower); f. *minor* Brot (= var. *depressa* Boiss., = var. *humilior* car et St. Lag.; stems with denser foliage only 4 – 15 cm tall, leaves smaller, flowers only terminal and smaller than usual); var. *diffusa* Grisebach (stems decumbent at the base, many flowers, leaves broadly ovate-lanceolate); f. *albiflora* Murr. (white corollas); f. *roseiflora* Zinserling (pink or lightly violet corollas). In addition, SIMMONDS (1946) described variability in size, shoot number, habit, leaf shape, and flower number and colour. He also discussed some evidence that these "varieties" are mere forms created under different abiotic conditions.

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* See pictures at the color plates.

2. Distribution and habitat requirements

2.1. Geographical distribution

Gentiana pneumonanthe occurs mainly in the temperate zone of Europe and Asia (Fig. 1). Its range includes West, Central, and Eastern Europe through Central Russia to the Urals, Siberia and Kamchatka, and south to the Caucasus, the mountains of central Asia and the Baikal region. In Europe the species grows in all countries of Western and Central Europe and the Balkans, excluding Greece. Southern and western European limits are in Portugal at about 9° W 38° N and northern limits are 59 ° 15′ N in Norway to about 61° N by Lake Ladoga in Russia (SIMMONDS 1946).

Kamčatka
není v mapě

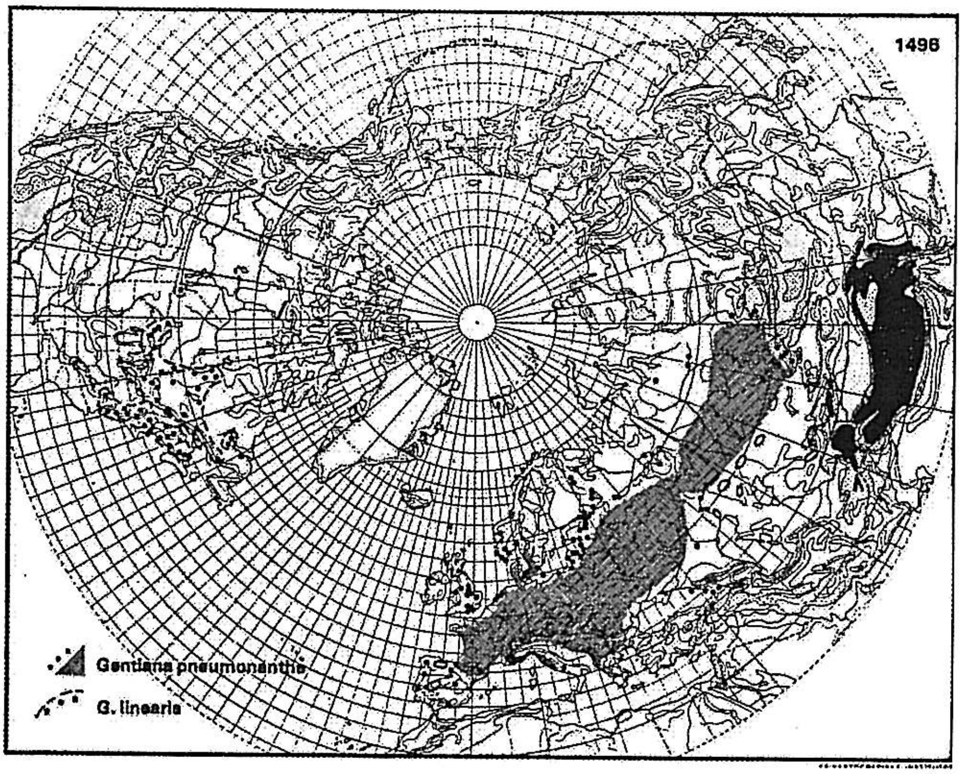

Fig. 1. Distribution of *Gentiana pneumonanthe* (HULTÉN & FRIES 1986).

2.2. Habitat

G. pneumonanthe is a lowland species rarely growing above 1200 m in Europe (HEGI 1975). In Central Europe unfertilised oligotrophic or mesotrophic wet meadows and extensive pastures, often with fluctuating underground water levels, are typical habitats of *G. pneumonanthe*. Soils are wet/podsols or gley that dried up in summer. Soil pH varies from neutral to moderately alkaline. Occasionally the species can be found in young/open pine and alder forests. The habitat is different in the Atlantic part of Europe, including the British Isles, where *G. pneumonanthe* occurs in wet acid heathlands (KESEL & URBAN 1999), on the borders of peat bogs (SIMMONDS 1946, CHAPMAN ET AL. 1989), and in wet unmanaged grasslands and hay meadows (OOSTERMEIJER ET AL. 1992, 1994B, 1998).

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/dry

2.3. Communities

Gentiana pneumonanthe is a diagnostic species of the phytosociological alliance *Molinion* (OBERDORFER 1962). Vegetation relevés from more than fifty recently sampled localities of *G. pneumonanthe* in SW Czech Republic were analysed by Twinspan and two main vegetation types were distinguished. Most populations grow in tall-herb meadows dominated by *Molinia coerulea*. It is difficult to classify community types exactly because of successional changes in abandoned hay meadows (Fig. 2). Community structure of the vegetation at other localities corresponds with the *Violion-caninae* alliance. The main vegetation types, their synecology and management are in Table 1.

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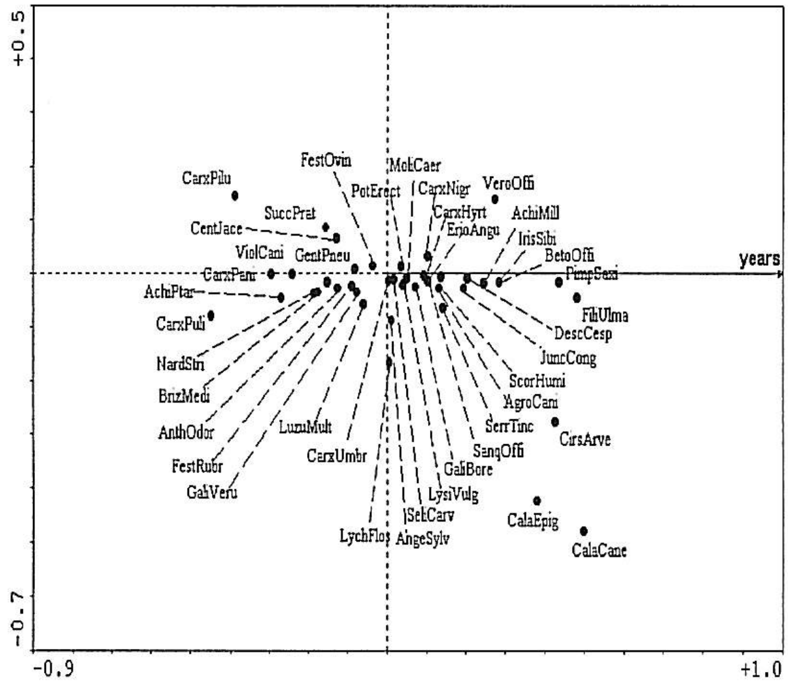

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Fig. 2. Diagram of Canonical Correspondent Analyses (TER BRAACK & ŠMILAUER 1998) – correlation between vegetation structure and a time since abandonment of sites as only explanatory variable.

Abbreviations: AgroCani - *Agrostis canina*, AchiMill - *Achillea millefolium*, AchiPtar - *Achillea ptarmica*, AngeSylv - *Angelica sylvestris*, AnthOdor - *Anthoxanthum odoratum*, BetoOffi - *Betonica officinalis*, BrizMedi - *Briza media*, CalaCane - *Calamagrostis canescens*, CalaEpig - *Calamagrostis epigeos*, CarxHyrt - *Carex hirta*, CarxNigr - *Carex nigra*, CarxPani - *Carex panicea*, CarxPilu - *Carex pilulufera*, CarxPuli - *Carex pulicaris*, CarxUmbr - *Carex umbrosa*, CentJace - *Centaurea jacea* CirsArve - *Cirsium arvense*, DescCesp - *Deschampsia ceaspitosa*, ErioAngu - *Eriophorum angustifolium*, FesrOvin - *Festuca ovina*, FestRubr - *Festuca rubra*, FiliUlma - *Filipendula ulmaria*, GaliBore - *Galium boreale*, GaliVeru - *Galium verum*, GentPneu - *Gentiana pneumonanthe*, IrisSibi -*Iris sibirica*, JuncCong - *Juncus conglomeratus*, LuzuMult - *Luzula multiflora*, LychFlos - *Lychnis flos-cuculi*, LysiVulg - *Lysimachia vulgaris*, MoliCaer - *Molinia caerulea*, NardStri - *Nardus stricta*, PimpSaxi - *Pimpinella saxifraga*, PoteErec - *Potentilla erecta*, SanqOffi - *Sanquisorba officinalis*, ScorHumi - *Scorsonera humilis*, SeliCarv - *Selinum carvifolia*, SerrTinc - *Serratula tinctoria*, SuccPrat - *Succisa pratensis*, VeroOffi - *Veronica officinalis*, ViolCani - *Viola canina*.

Table 1: Description of the plant communities as classified by Twinspan, together with their characteristic species, synecology, and management.

Communities of the *Molinion* alliance	**Synecology / management**
• Type with *Succisa pratensis* and *Selinum carvifolia* • Type with *Betonica officinalis* and *Galium verum*	Regularly mown hay meadows (every 1-5 years).
• Type with *Festuca rubra* and *Nardus stricta*	Unmanaged meadows on neutral to slightly acid soils; species poor - often surrounded by forest.
• Type with *Deschampsia ceaspitosa* and *Juncus conglomeratus*	Long-term unmanaged meadows on wet or dried up acid soils.
• Type with *Calamagrostis epigeos* and *Cirsium arvense*	Long-term unmanaged meadows strongly influenced from nutrient-rich neighbourhood (often fields).
• Type with *Carex dioica* and *Parnassia palustris*	Regularly mown hay meadows (every 1-3 years) on neutral to basic soils.
• Type with *Serratula tinctoria* and *Iris sibirica*	Unmanaged meadows on slightly basic loam.
Communities of the Violion-caninae alliance	
• Type with *Nardus stricta, Calluna vulgaris* and *Thymus pulegioides*	Irregularly managed pastures and dry meadows on rocky soils.
• Type with *Deschampsia ceaspitosa, Calamagrostis epigeos* and *Frangula alnus*	Long-term (several decades) abandoned pastures and dry meadows on forest edges.

2.4. Response to abiotic factors

Flooding after strong rains in region with watertight soils and ~~a~~ capillarity of an underground water are important factors. Well rooted adults as well as seedlings of *G. pneumonanthe* can survive several days/weeks under water level. Submerged plants continue the growth of buds. Flowers usually open immediately after a decrease in water levels. Extremely long submersion causes a reduction in growth and/or putrefaction of flowers and leaves. Indirect flooding effects are mechanical disturbance of the soil surface and suppression of competitors – gaps are essential for seedling recruitment (OOSTERMEIJER ET AL. 1994B, KŘENOVÁ & LEPŠ 1996). In Central Europe flooding is irregular at many localities. Flooding is more important in NW Europe, where annual fluctuations in water level appear to be essential for the creation of suitable open places in heathlands for recruitment (KESEL & URBAN 1999).

Burning is also more significant in NW Europe, especially in the southern British Isles, where heathlands are regularly burned (CHAPMAN & ROSE 1982, CHAPMAN ET AL. 1989). In Central Europe marsh gentian localities were burned intermittently to clear shrubs from extensively used pastures. Wet hay meadows were not burned.

2.5. Abundance

G. pneumonanthe is a rare species and its abundance in wet oligotrophic meadows is rarely greater than several percent of the vegetation cover. Meadow vegetation structure, management, and location safe sites for regeneration influence the abundance.

3. Life cycle and biology

3.1. Life cycle

Gentiana pneumonanthe is a hemicryptophyte that may live several decades. Individuals more than thirty years old are known (OOSTERMEIJER ET AL. 1994B, ROSE ET AL. 1998). There are no techniques for the non-destructive determination of an individual's exact age. Based on demographic studies six different age stages in marsh gentian populations were distinguished: seeds, seedlings, juveniles, vegetative adults, generative adults, and dormant (OOSTERMEIJER AT AL. 1994B). Seedlings have a cotyledon, one or two leaf-pairs, and do not have a stem or over-wintering shoot. Juvenile plants have one or rarely more stems and/or overwintering shoots, often carry cotyledon. Their leaves are thinner and usually more ovate than in adults. In open vegetation they are very short (0,5-2 cm) and in tall vegetation they are rather elongated (>10 cm). Vegetative adults are individuals without cotyledons, with one or more stems and have overwintering shoots in late summer. Their leaves are thicker and mostly revolute. Stems do not bear any flowers. Generative adults have, in addition to vegetative stems, one or more flowering stalks and overwintering shoots in late summer. Leaves are similar to those of the

vegetative adults. Dormants are individuals in a dormant state, with no aboveground organs.
A typical development sequence for a marsh gentian individual is from seed to a generative adult (Fig. 3). The period of time of an individual's persistence in each ontogenetic stage varies and is influenced by biotic and abiotic conditions. Generally plants remain one year in the seedling stage and one year in the juvenile stage, but flowering during the second year of life does occur.

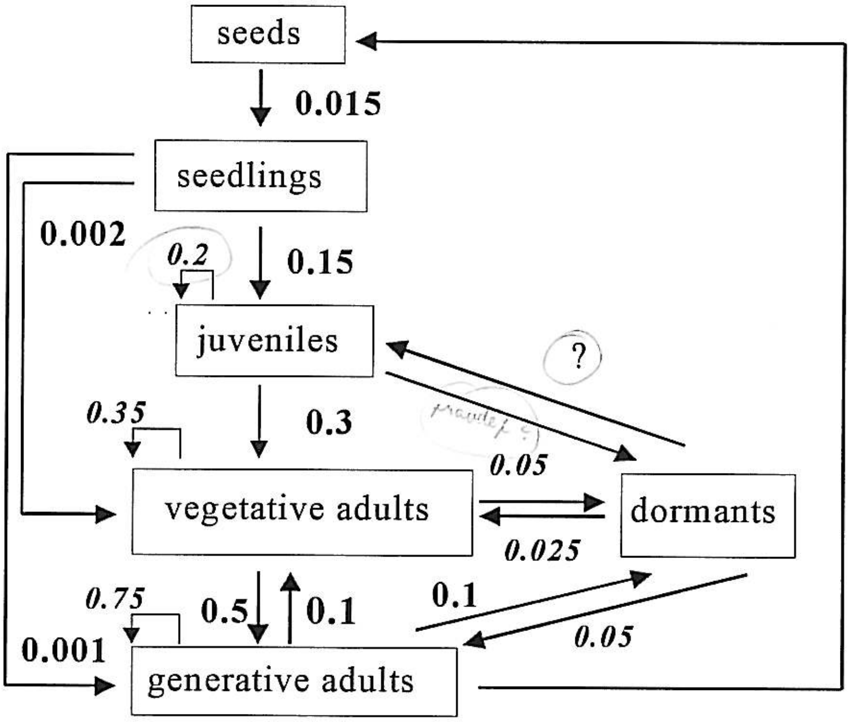

Fig. 3. The life cycle of *Gentiana pneumonanthe*. Arrows indicate transition probabilities. Bold numbers are probabilities known from monitoring and field experiments, numbers in italic are estimations. Time interval is one year.

Many individuals never flower, never reaching the generative adults stage. Retrogression also occurs and individuals may return to the vegetative stage after several years of flowering. Some individuals live in dormant stage through several years and create under-ground organs only. This usually occurs when environmental conditions are difficult. Many "new" adults may appear in long abandoned locations after a habitat revitalisation. In addition, sometimes it is impossible to discriminate dormants from individuals totally browsed by animals or fully covered by litter in unmanaged meadows.

The morphological structure of underground organs brings a better estimation of an individual's age. The number of mesocorm nodes is correlated with an individual's age. New adventitious roots grow horizontally from the highest part of the mesocorm every year and a new crown of stalks and overwintering shoots runs up from the top. This is way how the long-lived *G. pneumonanthe* individuals keep on a surface in habitats where several centimetres of organic matter is accumulated every year. /are

3.2. Spatial distribution of plants within populations

The local spatial distribution of *G. pneumonanthe* depends on vegetation structure, disturbance intensity and management regime. Disturbance history is very important. A spatial clustering of individuals corresponds with a distribution of safe sites over several years. At long abandoned meadows marsh gentians are clumped only in locations where irregular disturbances (e.g., wild animals, flooding, etc.) inhibit succession changes and create gaps essential for reproduction.

3.3. Phenology

Seeds of *G. pneumonanthe* germinate from the end of March to June. Occasionally a small proportion of the seeds germinate immediately after dispersal in autumn. Stems usually appear during May or the beginning of June. Flowering commences in July and peaks during the first weeks of September. Seeds ripen in September and October. The creation of over-wintering shoots starts at the end of August. Different climatic, soil and hydrological conditions cause small variations in phenology among populations. Also, seasonal variability in management stimulates phenological advances. Later flowering can be observed at locations that are regularly mown at the beginning of summer. Faster ripening of seeds occurs at locations grazed in late September and October. Hot and dry summers reduce the flowering season to two or three weeks and cold and wet autumns elongate the time required for the ripening of seeds.

3.4. Reproduction

Gentiana pneumonanthe reproduces only by seeds. Protandrous flowers of *Gentiana pneumonanthe* are pollinated by bumblebees after nectar. Also, pollination by bees and some other insects (zygaenid moths) can not be ruled out. Species self-compatibility has been verified in field manipulation experiment conducted in the species rich meadow, where the large population of *Gentiana pneumonanthe* occurred (Table 2, Fig. 4).

Marsh gentian pollination biology in wet heathlands has been studied in the Netherlands (PETANIDOU 1991, PETANIDOU ET AL 1995, 2001). results ?

Seeds (usually about 300 – 700 (1000) per capsule) are very small, ovate, and nearly unwinged. They are transported mechanically or by wind only small distances immediately after maturation. Some seeds remain in the opened capsules and come off later.

Table 2. Effect of mowing surrounding vegetation and type of pollination or reproduction of *G. pneumonanthe*. Results of ANOVAs are presented, $p < 0.05$ are bold. For more details see the legend of Figure 4.

	Number of viable seeds		Seed-set		Weight of seeds and ovules	
	F	p-level	F	p-level	F	p-level
Mowing of surrounding vegetation	1.521	0.222	1.001	0.321	0.829	0.365
Type of pollination	3.330	**0.042**	4.635	**0.013**	4.498	**0.015**
Interaction: type of poll. x mowing	0.651	0.525	2.342	0.104	0.850	0.432

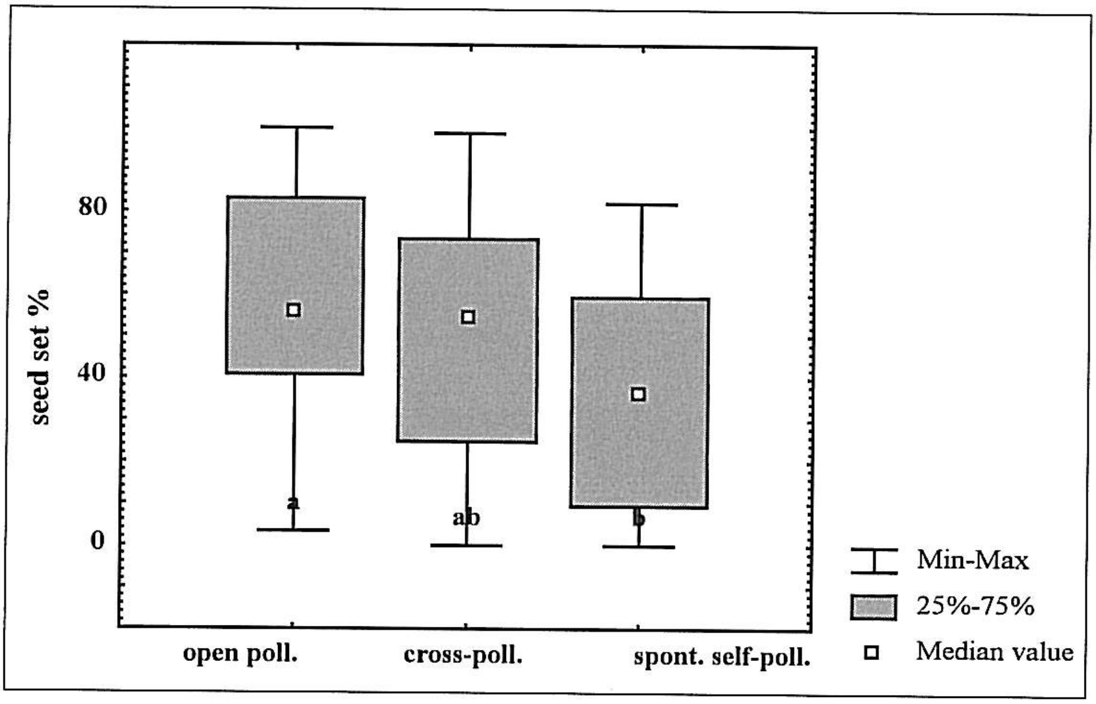

Figure 4. Seed set (a proportion of viable seeds from ovules per a capsule) in capsules under various pollination treatments. Treatments labeled with the same letter do not differ significantly (P< 0.05). Self-compatibility of *Gentiana pneumonanthe* was tested in a large gentian population (about 500 flowering individuals).

Design of experiment: Sixty adult gentians were chosen randomly and marked at the beginning of flowering period. Three treatments were applied to each individual: one flower was bagged to prevent insect visitation, the second one was emasculated (but pollination from other flowers of the same individual wasn't excluded), and the third flower was only marked and left as a control. The vegetation was clipped out to 50 cm around 30 individuals to test for an influence of surrounding vegetation. Significant differences in seed production, seed-set, and weight of seed and ovules (P< 0.05; Table 2) were found between bagged flowers and control open-pollinated flowers. No significant differences between plants growing in open sites and in dense vegetation were found (KŘENOVÁ & LEPŠ 1996).

3.5. Germination

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Seeds germinate instantaneously in temperatures greater than 25° C. Cold and dry stratification (3-5 months) increases a germination rate (THOMPSON 1969). In laboratory conditions seeds of *G. pneumonanthe* germinate well on moist soil in sheltered locations. In addition, germination on wet filter paper is possible but budding seedlings are often contaminated by fungal infection. Seeds can also germinate on a sterile substrate. Seedling vitality without mycorrhizal inoculation is lower but they can survive in low competition situations. The best germination rate of about 35% was achieved under a 12 hour dark/light regime with temperatures of 8°C/20°C. Older seeds germinate slowly and erratically. In a laboratory experiment germination of two years old seeds was about 9% and germination of five years old seeds was only 4,5%. For this experiment seeds were stored in a refrigerator at 5°C. In spite this a seed bank probably doesn't play role in a reproduction cycle of *G. pneumonanthe*. (OOSTERMEIJER ET AL. 1994B).

In the field *G. pneumonanthe* is a typical "gap" species. This means that gaps, i.e., places without vegetation cover and preferably with open bare soil are essential for the initial phases of life. Several authors have attested that *G. pneumonanthe* seeds germinate well and seedlings survive successfully only in sites with open bare soil (OOSTERMEIJER ET AL. 1994B, KŘENOVÁ & LEPŠ 1996, KESEL & URBAN 1999). Field observation and several manipulation experiments in species rich meadows show that a germination rate of about 20-25% is followed by high mortality. More than 60% of the seedlings died in suitable microsites with cut sod and >85 % died in mown plots where small gaps occurred during first summer (Fig. 5; KŘENOVÁ & LEPŠ 1996).

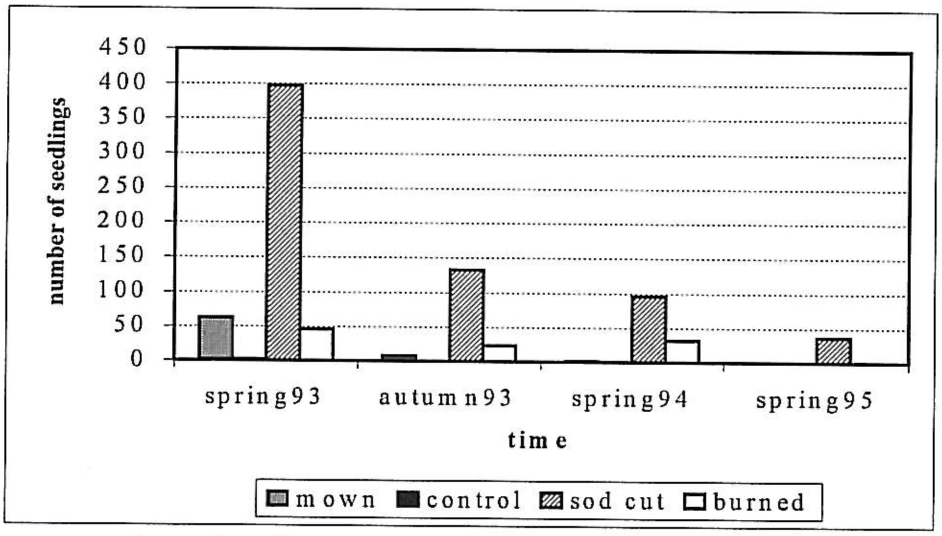

Figure 5. Average numbers of seedlings of *Gentiana pneumonanthe* survived in plots under various treatments (KŘENOVÁ & LEPŠ 1996). About 15000 seeds were sown in each 0.25 m^2 plot in early spring 1993. More than 60% of seedlings died in plots with sod cut where a germination rate was the highest and 85% of seedlings died in mown plots.

3.6. Response to competition and management

OOSTERMEIJER ET AL. (1994B) characterised the marsh gentian in unmanaged hay meadows as a "C-S-R-strategist" in sensu GRIME (1979). Adult individuals produce a high number of small seeds, which only colonise open patches in closed vegetation. Size, shape, and mechanisms of creation of favourable gaps differ in locations with different vegetation structure, cover, time of abandonment, etc. Small gaps (only several cm^2) are common enough in periodically mown hay meadows and pastures. In larger gaps seedlings overheat and dry out. However, in long abandoned grasslands large gaps are necessary, because the surrounding vegetation and litter in small gaps stresses seedlings and juveniles (KŘENOVÁ 2001- submitted). It is a different situation in heathlands, which were traditionally managed by large sod cutting. Depressions with open bare soils are periodically flooded and the seedling benefits from increased soil moisture.

Vegetative and generative adults can persist many years in closed vegetation. Individuals growing in long abandoned meadows are tall because of light competition with the tall and dense surrounding vegetation. Flowers are pollinated and seeds develop well but recruitment is limited. The gentian population can rejuvenate itself immediately after the vegetation is opened up. Individuals strongly shaded by surrounding vegetation are flattened and usually have only 1 or 2 stems. Their flowers are seldom pollinated and seed capsules are often infected by fungi or destroyed by herbivores. Population size under these conditions rapidly declines.

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Based on proportions of ontogenetic stages in populations three different population types can be distinguished: (a) - *invasive* or *dynamic* population, characterised by a high ratio of seedlings and juveniles to adult plants; (b) - *normal* or *stable* populations with a higher density of adults and fewer juveniles; (c) - *senile (senescent)* or *regressive* populations where only adult vegetative and generative plants exist (OOSTERMEIJER ET AL. 1994B). This classification was determined in the Netherlands where most *G. pneumonanthe* populations occur in heathlands. This classification must be adapted for Central Europe where the majority of the populations occur in oligotrophic hay meadows. *Invasive* populations, although rare, occur in locations with strong mechanical disturbance of the soil surface (for example in military training areas). *Normal* populations occur in traditionally managed meadows and pastures where regular management prevents closure of the vegetation and assures the creation of small-scale gaps that are essential for species regeneration. Adult gentians predominate and both seedlings and juveniles occur in low densities. Quantification of seedlings and juveniles in closed vegetation of species rich meadows is extremely difficult. *Senile* populations occur in abandoned meadows, old pastures, and spontaneously afforested locations. Several hundred or only a few individuals may occur in senile populations. Also, vitality of individuals varies and some populations are sentenced to extinction. During 1995-2000 factors affecting the vitality of more than fifty *G. pneumonanthe* populations were studied in SW of the Czech Republic (Fig. 6; Appendix 1).

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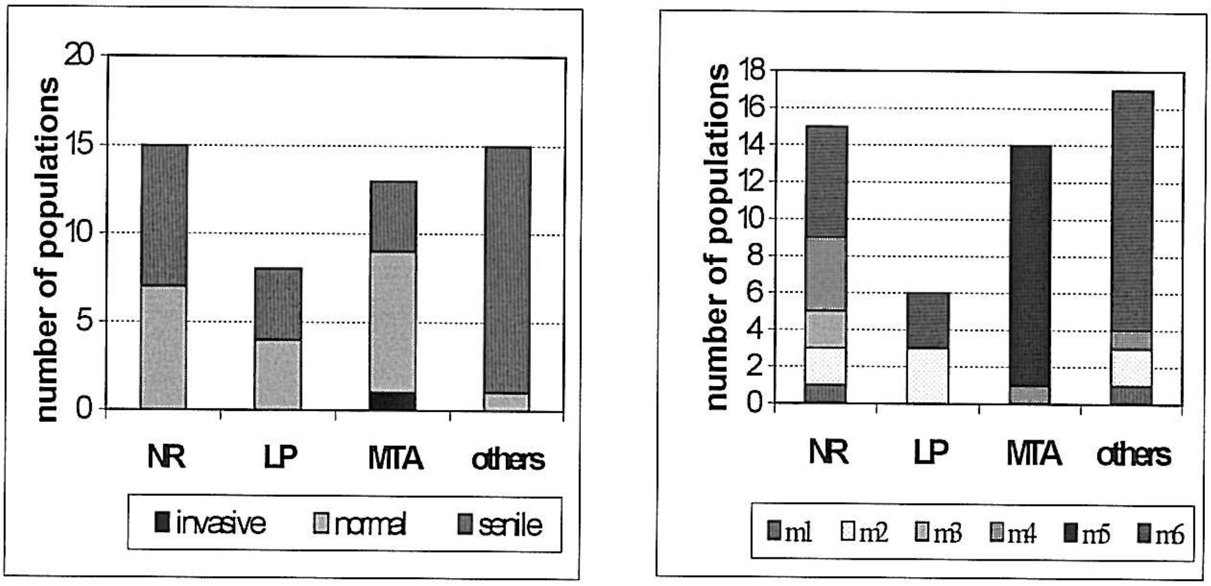

Figure 6. Proportion of three types of *Gentiana pneumonanthe* populations – invasive, normal, senile – at 50 various locations in SW of the Czech Republic, Central Europe.
Four categories of locations were distinguished: NR – nature reservations; LP – areas protected by local authorities; MTA – military training areas; others – unprotected areas etc. These categories are characterised by various types of management: m1 – pastures, m2 - by hand, m3 - meadows regularly mown by machinery, m4 – mix m2 and m3, m5 – irregular disturbances, m6 – unmanaged.

3.7. Herbivores and pathogens

Wild animals, especially deer, browse gentians at many locations where a high proportion of the flowering individuals may be destroyed by browsing. In addition, livestock, sheep, goats, and horses may browse gentians in pastures. In pastures where only few gentians occur most of them can be destroyed. In pastures with large populations sheep and goats browse only few gentians that stimulate their digestion. Cows are less dainty and it is better to exclude areas with flowering gentians from grazing. Early in the season herbivory stimulates the creation of new branches from adventitious buds and increases the number of flowers per plant. Later, when flower buds are well developed the creation of new branches and flowers by herbivory is limited. In addition, the ability of these post-herbivory flowers to produce seeds is very low. Small mammals (mouse, shrew-mouse etc.) also browse gentians. Slugs destroy a large number of seedlings and juveniles.

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Many insect species are herbivores of *G. pneumonanthe*. Larvae of *Stenoptilia graphodactyla* (Treitschke 1833) and *S. pneumonanthe* (Büttner 1880) feed on the flowers, young seed capsules, and leaves of *G. pneumonanthe*. The host plants of *S. graphodactyla* are several species of the *Gentiana* genus. Larvae of *S. pneumonanthe* were found only on *G. pneumonanthe* and *G. cruciata*. Pupae stay on stems of *G. pneumonanthe* or neighbouring plants for several weeks. Larvae of *Dasineura* *gentianae* (Kieffer 1909), which probably consume the parenchyma of the gynoecium were found. The most interesting herbivore of *G. pneumonanthe* is the myrmecophilous blue butterfly *Maculinea alcon.* Females of the species oviposit on buds and flowers of *G. pneumonanthe*. Caterpillars live inside gentian flowers for several weeks. When they reach the fourth instar the drop down and wait for *Myrmica* worker ants. The ants adopt them and carry them into an ant nest until the next summer. *Maculinea alcon* is a very rare species. Many authors (reviewed in Thomas et al. 1998) have studied the ecology and behaviour of this species. An occurrence of *Maculinea alcon* is strongly correlated ($p < 0.001$) with a population size of its host-plant *G. pneumonanthe* (Fig. 7, KŘENOVÁ & PAVLÍČKO 2001 – in prep.).

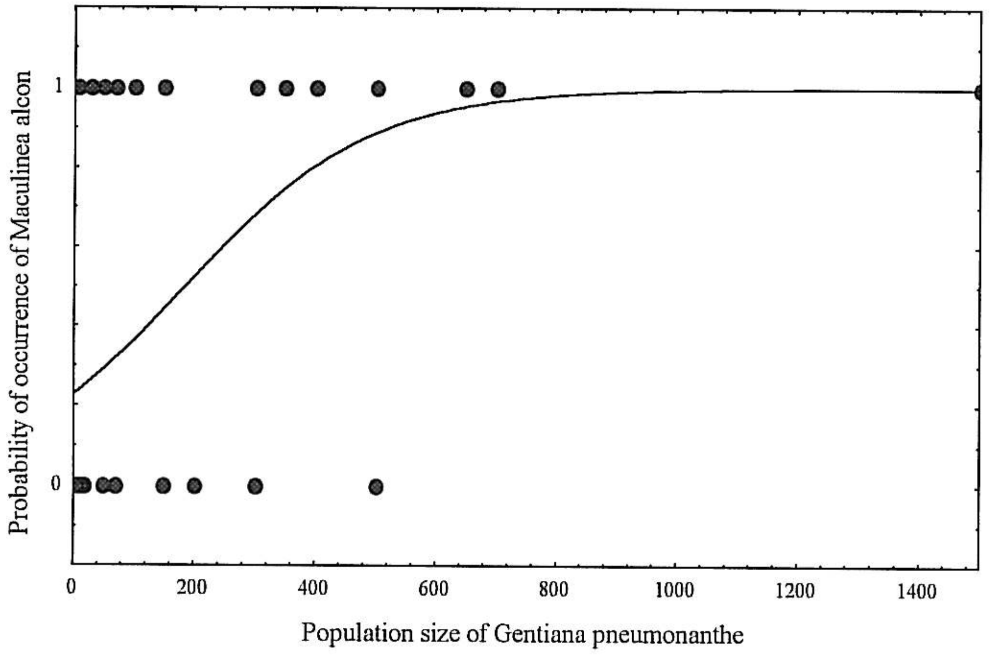

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Figure 7. Logistic regression – the population size (resp. the number of all flowering individuals) of the host-plant *Gentiana pneumonanthe* is a good predictor of *Maculinea alcon* occurrence ($p<0.001$; KŘENOVÁ & PAVLÍČKO 2001 – in prep.).

Seeds, seed capsules and occasionally anthers may suffer from fungal infection. Fungal infection is more common during rainy summers and in flowers of flattened plants. In extreme wet conditions a high proportion of seeds can be destroyed by mould. Sometimes individuals with morphologically untypical stems can be found with twisted stems, foliage condensed to one side, and undeveloped flowers. These deformations will appear on the same individuals every year.

3.8. Mycorrhiza

There is no detailed information about the mycorrhizae of *G. pneumonanthe* but studies show that the basic knowledge of the mycorrhizae of other gentians may be applied to *G. pneumonanthe*. ? *Gentianaceae* species show different intensities of mycorrhizal colonisation. In some species no symbiosis could be observed. /y Arbuscular mycorrhiza (AM) was found in some species (GAY ET AL. 1982, JACQUELINET-JEANMOUGIN & GIANINAZZI-PEARSON 1983, MC GEE 1985). Gentians successfully germinate without mycorrhizae (Jacquelinet-Jeanmougin & Gianinazzi-Pearson 1983 and several other authors) and subsequent growth appears to be enhanced when the roots are not colonised by AM fungi (MC GEE 1985). TO JE DEFINICE PRO "AM fungi" Zygomycetous fungi belonging to the order Glomales infect roots of *G. pneumonanthe*. AM fungi in gentians show intracellular growth from cell to cell called "structural incompatibility" ?? and more additional infections of hyphae are essential for continuous growth of gentian roots. These hyphae propagate from the roots of surrounding plants (WEBER 1993). Mycorrhizae of *Gentiana* species colonize other plants (e.g., *Cirsium vulgare* or *Plantago lanceolata*; DEMUTH ET AL. 1989).

3.9. Physiological data

No information available.

3.10. Biochemical data

Evidence for the production of different types of secondary compounds is only circumstantial and based on findings in other *Gentiana* species. Some of them (above of all *G. lutea*) have been used by healers since the Middle Ages. Secondary metabolism of *Gentiana* species produces glycosides, xanthones, flavones, and secoiridoids. A new C –13 glycoside was found in *G. pneumonanthe* (MPONDO ET AL. 1989). Root extracts, which are now components of some drugs, stimulate digestion, irritate mucous membranes, and in part kill the plasmodium that causes malaria. These drugs are not recommended for pregnant or breastfeeding women and infants.

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3.11. Genetic data

Cytology: The basic chromosome number is 26. No polyploids have been found (KIRSCHNER & KIRSCHNEROVÁ 2000).

Genetic variation: Genetic variation of *G. pneumonanthe* has been studied in many populations in several countries (OOSTERMEIJER ET AL. 1995A,C). In harmony with population genetic theory it was ? found that small populations have a reduced level of genetic variation (isoenzyme analyses were done) and their present isolation has resulted in very limited interpopulation gene flow level (RAIJMANN ET AL.

1994). Also, the relation between population size and genetic variation, allozyme heterozygosity and fitness, and inbreeding depression and local adaptations in small and large populations have been studied (OOSTERMEIJER ET AL. 1994A, 1995A, B, C). Study of genetic variation of *G. pneumonanthe* populations in Central Europe is in progress and results only for four populations are ready. All study /ied populations are in the Hardy-Weinberg equilibrium and comparison of results with results of genetic studies from various parts of Europe showed that genetic variation of *G. pneumonanthe* populations in Central Europe is higher (Table 3).

Table 3. Genetic variation of *Gentiana pneumonanthe* populations in various parts of Europe. Populations – number of studied populations (in the Czech Republic the project is in progress and only preliminary results are available), locus – number of studied polymorphic locus, Ap - average numbers of effective allelas per locus, Ho-p a He-p - averages of the observed and predicted proportion of heterozygotes in populations.
*RAIJMAN ET AL. 1994, ** OOSTERMEIJER ET AL. 1995C, *** MULDER – unpublished data.

	Populations	**Locus**	**Ap**	**Ho-p**	**He-p**
Czech Republic	4	3	1.38	0.452	0.473
Netherlands *	25	16	1.21	0.119	0.133
Norway **	14	6	1.15	0.042	0.046
Great Britain ***	5	12	1.07	0.011	0.017

3.12. Hybrids

No natural hybrids are known.

3.12. Status of the species

Before the 1960's, *Gentiana pneumonanthe* was common and locally widespread. In all of Central and Western Europe both the number of populations and the size of populations rapidly declined as a result of habitat loss and land use changes. Large areas of wet oligotrophic meadows, very species rich communities, were drained and/or fertilised and thereby changed to species-poor meadows (often monocultures). Traditional management practices have been considered uneconomical and meadows abandoned. Landscapes have been fragmented and the species survive only in small and isolated populations.

Gentiana pneumonanthe is protected and included in the Red Lists of Flora of many European countries. The species now occurs principally in Nature protected areas, some regions which preserve traditional management practices, and military training areas (i.e., areas inhabited in the middle of the

20th century without intensive agriculture management but with occasional disturbance activities that block succession).

Acknowledgements

The research was supported by the Grant Agency of the Czech Academy of Sciences (junior grant GA AV ČR 6141901), GAČR 206/99/0889, FRVŠ 0334, and the Mattoni Award. I would like to thank my supervisor Jan Lepš, Marie Šmilauerová, Milan Štech, and Jitka Klimešová for their comments and to Jonathan Titus for his comments and correcting English. I am grateful to many amateur botanists, local managers, and conservation authorities, who inform me about the gentians populations.

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Appendix 1

Biotic and abiotic characteristics of 52 study* Gentiana pneumonanthe *sites in SW part of the Czech Republic.

* - populations of *Gentiana pneumonanthe* extinct during study period 1994 – 2000 and were not included in analysis;
GP94-2000 – number of flowering individuals of *G. pneumonanthe*; Poptype – type of *G. pneumonanthe* population, 1 – invasive, 2 – stabile, 3 – senile; Height – average height of stems [cm]; Stem – avarage number of stems per one individual; Flower - average number of flowers per one individual; E% - total % cover; Eo% - % cover of mosses; Area - site area [ha]; Protection - 1– natural reservations; 2 – areas protected by local authorities; 3 – military training areas; 4- others – unprotected areas; Management: 1 – pastures, 2 – mowing by hand, 3 - meadows regularly mown by machinery, 4 – irregular disturbances, 5– unmanaged.

	GP94	GP95	GP96	GP97	GP98	GP99	GP2000	Poptyp	Height	Stem	Flower	E%	Eo%	Area	Protection	Management
1	.	10	10	10	10	12	12	3	36	2.7	1.7	90	10	0.1	2	5
2	.	.	16	.	.	10	10	3	53.9	3	3.5	95	5	0.4	4	5
3*	.	.	0	.	.	0	0	.	.	.	.	.	.	.	4	.
4	.	.	1	.	.	1	1	3	48	13	8	85	20	0.1	1	5
5	.	.	1	.	.	1	1	3	48	12	9	70	20	0.02	1	5
6*	.	.	3	.	.	0	0	.	.	.	.	.	.	.	1	.
7	.	.	300	.	.	1500	1300	2	38.1	2.5	15.2	95	5	2.4	1	2
8	600	600	600	500	100	300	150	2	46.5	2.3	15.3	95	5	1	1	2
9	4	5	8	5	6	7	15	3	58.8	3.7	21.2	95	15	0.75	4	4
10	.	21	25	15	.	10	10	2	44.9	2.7	7.2	90	10	1	1	5
11	20	20	8	7	7	7	.	3	38.1	2.4	5.6	95	2	0.01	4	5
12	50	50	50	50	65	50	60	3	52.9	3.2	24	98	1	1.2	1	5
13*	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.
14	.	80	200	300	700	600	500	2	39.5	3.3	13	98	4	2	1	3
15	.	50	100	100	150	150	90	2	55.7	3.5	23.1	98	5	0.8	4	5
16*	.	.	6	6	.	1	.	.	.	.	.	.	.	.	1	.
17	.	.	.	.	150	150	150	3	36.6	3.6	28.4	85	10	0.5	1	2
18	.	.	200	250	200	200	200	2	34.5	2.5	6.9	97	15	1	1	3
19	300	300	350	400	500	450	450	2	35.9	2.7	5.1	75	40	3	1	3
20	.	.	400	300	300	300	300	2	45.3	3.7	8.8	85	50	1	1	3
21	.	.	200	200	150	150	50	2	39.4	2.7	9.6	98	3	5	3	4
22	.	.	100	100	150	150	150	2	46.6	2.2	11.9	90	7	1.5	3	2
23	.	.	60	6	25	20	12	3	49.7	1.6	6.1	95	0	1	3	4
24	.	.	350	350	500	450	550	2	53.9	3.6	16.5	90	10	1	3	4
25	.	.	6	.	.	6	.	3	54	4.I	23.3	95	15	0.5	1	1
26	.	.	1	2	1	1	.	3	48	2	7	95	5	1	4	5
27	.	.	1	1	1	1	1	3	60	1	8	85	1	0.75	1	2
28	.	.	500	500	550	550	250	2	37.3	1.8	5.5	90	10	7	3	4
29	.	.	5	.	4	5	5	3	39	5.5	15.5	95	20	0.5	3	4
30	.	.	150	100	100	100	150	2	54.3	3.7	9.9	95	5	1	3	2
31	.	.	4	4	3	3	.	3	32.5	4.5	5	98	4	0.01	3	5
32	.	.	50	43	38	40	25	2	37	4	6	95	5	0.4	3	4
33	.	.	.	100	100	100	80	2	13.1	2.9	4.7	85	10	2	4	1
34	.	.	.	.	45	43	12	1	34.8	1.5	4.1	70	50	0.05	3	4
35	.	.	.	.	8	8	.	3	46.4	2.8	10	85	10	0.01	4	5
36	.	.	.	.	4	4	.	3	46.8	4.6	31.2	95	3	0.05	4	5
37	.	.	.	.	350	450	450	2	32.5	2	14.5	85	0.5	5	2	2
38	.	.	.	.	300	300	100	2	46.4	2.4	23.6	90	5	3	2	2
39	.	.	.	.	1	1	1	3	72	9	33	95	7	3	4	5
40	.	.	.	.	15	15	15	3	49.1	2.7	22.9	97	3	0.01	1	5

Appendix 1 – continued.

	GP94	GP95	GP96	GP97	GP98	GP99	GP2000	Poptyp	Height	Stem	Flower	E%	Eo%	Area	Protection	Management
41	.	.	.	.	.	80	50	3	30.1	4.9	2.1	90	20	0.05	4	5
42	.	.	.	.	.	700	750	2	35.9	2.4	8.9	98	50	2	2	2
43	.	.	.	.	.	500	500	3	44.4	3.6	42.4	98	2	2	4	5
44	.	.	.	.	.	5	5	3	42	2.6	0.2	75	0.5	0.2	2	5
45	.	.	.	.	.	70	50	3	52	2.1	9	90	5	1	2	5
46	.	.	.	.	.	20	20	3	44.2	5.4	14	95	5	1	2	5
47	.	.	.	.	.	70	80	3	53.1	6.1	17.2	97	20	2	2	4
48	.	.	.	.	.	60	60	3	56	3	15.2	98	15	3	4	5
49	.	.	.	.	.	2	2	.	.	.	.	.	.	.	3	.
50	.	.	.	.	.	6	6	3	37	2	21.8	98	2	0.2	3	.
51	.	.	.	.	.	.	3	3	20.3	4.7	3.6	95	5	0.1	4	5
52	.	.	.	.	.	.	50	1	22.3	3.1	6.1	85	2	3	3	4

Chapter 3

Regeneration of a *Gentiana pneumonanthe* population ~~a~~ in an oligotrophic wet meadow

Journal of Vegetation Science 7: 107-112, 1996
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Regeneration of a *Gentiana pneumonanthe* population in an oligotrophic wet meadow

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Abstract. The aim of this study was to evaluate the critical phases of the life cycle of *Gentiana pneumonanthe*, the marsh gentian, a threatened species of the Bohemian flora. The effect of various conditions on germination and seedling establishment and the possible effect of competition on the performance of the species were tested. Seeds were sown in plots which were subjected to four treatments in a randomized complete blocks experiment: unmanaged meadow, mown meadow, burned meadow, and meadow with cut sod. The significantly highest recruitment was found in plots with cut sod, the lowest in the unmanaged control plots. Seedling survival also differed among the treatments. In the following year surviving individuals were only found in plots with cut sod. The influence of neighbouring vegetation on target gentian individuals was evaluated by removing the surrounding vegetation and comparing the performance of these individuals with controls. The initial height of each individual was measured and used as a covariable. No significant effect of neighbouring vegetation on performance was detected. Thus, the establishment phase appears to be critical for population persistence and is also more influenced by the management regime than other stages of the life cycle.

Keywords: Conservation biology; Critical phase; Disturbance; Gap; Management: Seedling recruitment.

Nomenclature: Rothmaler (1976).

Introduction

Mechanisms for the maintenance of species diversity and the role of disturbance in this process are topical issues of contemporary community ecology (e.g. Grime 1979; van der Maarel 1993; Palmer 1994). Those issues are not only of academic interest, but also extremely important for management plans for plant communities in nature conservation. Data on the biology of rare species are crucial for successful management, together with the recognition of the critical stages of plant development necessary for proper management practices. The species of our concern, *Gentiana pneumonanthe*, has been declining throughout Europe (Hvatum 1993; Oostermeijer et al. 1992). Reduction of populations of *Gentiana pneumonanthe* has seriously influenced dependent insects as well, notably the rare butterfly *Maculinea alcon* which has disappeared from many localities (Elmes & Thomas 1992).

For many perennial species, the successful establishment of a new genet is the most critical step in their life history (see the concept of 'regeneration niche', Grubb 1977, and the carousel model of van der Maarel & Sykes 1993 for the community consequences). Transient gaps in the turf are instantly colonized and occupied, while new gaps are being created elsewhere (Rusch 1988; Rusch & van der Maarel 1992). Gap dynamics is an important mechanism for the maintenance of diversity in grasslands (Grubb 1977; Rusch 1988; van der Maarel & Sykes 1993). Tilman (1993) considered inhibited germination and/or survival of seedlings in productive grasslands to be a major cause of decrease in species richness along a productivity gradient.

In central Europe, meadows at lower altitudes are man-made and depend upon continuing human intervention. Traditional management practices, mainly grazing and cutting (Bakker 1989), have maintained these meadows for centuries, but recently the management practices have changed dramatically. Management was either intensified by fertilization, or the meadows were abandoned because they were no longer profitable. Both processes produce changes in species composition which are usually accompanied by extirpation of some species and overall loss of species diversity. At present, this trend can be exemplified with *Gentiana pneumonanthe* in most of its European localities. Regeneration in abandoned meadows is limited, as only adult plants survive, where the populations become senile (Oostermeijer et al. 1994b). However, it has been shown that new plants establish following disturbance (Chapman & Rose 1982; Chapman et al. 1989).

The aim of our study was to experimentally test the importance of various management and disturbance practices, including creation of artificial gaps in the turf for the regeneration of *Gentiana pneumonanthe* and to evaluate the effects of neighbouring vegetation on the performance of established plants. In this manner, the sensitivity of various stages of the life history of *Gentiana pneumonanthe* to influences of the neighbouring vegetation was compared and the critical phases of the plant life cycle determined.

Material and Methods

Study species

Gentiana pneumonanthe L., the marsh gentian, is a long-lived perennial species, which passes through the winter as a rosette of short shoots with very small leaves. The species occurs in wet heathlands and moist oligotrophic to mesotrophic hay meadows across temperate Europe and Asia, ranging in Europe from southern Scandinavia to the Balkans and northern Spain (Simmonds 1946). Reproductive adult plants can reach 50 cm in height. They usually have 1 - 10 stems, each stem bearing 1 - 25 flowers per year. The seeds (usually 300 - 700 per capsule) ripen during September and October. They are small and light (0.32 mg on average), and contain very little endosperm (Salisbury 1942; Petanidou et al. 1991; Petanidou et al. 1995; unpubl. data).

In the Czech Republic, *Gentiana pneumonanthe* occurs at the margins of peat bogs and in wet oligotrophic meadows, mainly in the piedmont. The species was previously quite common on traditionally managed meadows, but many populations decreased in size or became locally extinct during the last decades. At present, the majority of the populations survive in small isolated habitat islands, with low and declining population sizes. This imposes a further threat to their genetic diversity (Oostermeijer et al. 1994a; Raijmann et al. 1994; Lande 1988). A similar decline has been recorded for marsh gentian populations in Norway (Hvatum 1993), the United Kingdom (Chapman & Rose 1982) and the Netherlands (Oostermeijer et al. 1992a,b; Schaminée et al. 1995), with a likely similar pattern through all of central and western Europe.

Study site

All observations and field experiments were carried out at Ohrazení, near České Budějovice, southern Bohemia, Czech Republic. This site contains one of the largest remaining populations in Bohemia, with several hundreds of mature *Gentiana pneumonanthe* plants.

Here we are dealing with a wet oligotrophic meadow of ca. 1 ha in size, situated between a field and a pine-oak wood that was abandoned five years ago (some of the wetter parts even earlier). The unfertilized meadow community belongs to the *Molinion* alliance according to the Zürich-Montpellier phytosociological classification. It is characterized by the following relevé on a representative plot of 5 m × 5 m:

Cover of the herbaceous layer: 95 %. Species (first graminoids, then broadleaved herbs, both groups in alphabetical order) with their cover-abundance according to the Braun-Blanquet cover-abundance scale:

Anthoxanthum odoratum	2	*C. pulicaris*	+
Avenochloa pubescens	1	*Cynosurus cristatus*	1
Briza media	2	*Festuca rubra*	2
Carex flava s.l.	+	*Holcus lanatus*	1
C. hartmanii	1	*Juncus effusus*	1
C. leporina	+	*Luzula multiflora*	1
C. nigra	1	*Molinia caerulea*	2
C. pallescens	1	*Nardus stricta*	2
C. pilulifera	1		

Betonica officinalis	1	*Potentilla erecta*	1
Cirsum palustre	+	*Ranunculus acris*	+
Dactylorhiza majalis	+	*R. auricomus*	1
Galium boreale	2	*R. nemorosus*	+
Gentiana pneumonanthe	+	*Rhinanthus minor*	+
Leontodon hispidus	1	*Scorzonera humilis*	+
Leucanthemum vulgare s.l.	r	*Selinum carvifolia*	2
Lychnis flos-cuculi	1	*Serratula tinctoria*	+
Lysimachia vulgaris	+	*Succisa pratensis*	1
Myosotis palustris s.l.	+	*Valeriana dioica*	1
Plantago lanceolata	+	*Viola canina*	1

Seedling establishment

Seedling recruitment was evaluated under various conditions. Capsules of *Gentiana pneumonanthe* containing ripe seeds were collected in October 1992 and stored at 5 °C for three months. The experiment was arranged in five randomized complete blocks of 0.5 m × 0.5 m square plots. In January, aliquots of 0.6 g equivalent to 15000 - 20000 seeds, were sown in plots subject to the following four treatments:

(1) meadow after mowing: above-ground part of the biomass was cut off and the litter completely removed;

(2) burned meadow: the above-ground vegetation and the litter were burned, with the ash left in situ;

(3) meadow with cut sod: the sod was cut, about 10 cm deep, with the cut portion turned upside down;

(4) control: meadow was left in the original condition.

In all the treatments the seeds were sown by hand with extreme care to evenly cover the entire plot. However, edge density was slightly lower, but this should not have influenced the results as any effect was similar in all the treatments.

The number of emerging seedlings was counted only within the inner 0.25 m × 0.25 m plots to avoid edge effect. The first sampling in summer 1993 provided information about seedling emergence, with the following two (autumn 1993 and summer 1994) yielding data for evaluation of seedling survival. With regard to the biology of the species we expected that only a negligible fraction of seeds would germinate between the summer and autumn censuses. Our personal, non-quantified observations, showed that the majority of seedlings emerged in mid-spring. A similar behaviour was quantitatively described for *Gentianella campestris* by Milberg (1994).

A univariate repeated measures ANOVA (i.e. split-plot design applied to the repeated-measures data, von Ende 1993), reflecting the block structure of the experiment, was used to analyse the data. Generally, we can say that in the repeated measures analysis, the between-subject variation corresponds to differences between observed sampling units (block and treatment) and the within-subject variation corresponds to changes in time (see von Ende 1993 or any other appropriate statistical text for more detailed explanation). The numbers of seedlings were log-transformed – using log $(x + 1)$ to handle zeroes in the data – to achieve normality and homogeneity of variance. After log-transformation, the between-subject factor corresponds to differences in seedling establishment, and the interaction term between treatment and time corresponds to differences in seedling survival. Provided that the seedling survival is independent of the treatment, the number of seedlings in a plot after a time interval is $N_2 = p N_1$ in all plots, where N_1 and N_2 are numbers of seedlings in a plot in respective periods and p is the common probability of survival. Then $\log N_2 = \log p + \log N_1$, i.e. the factors treatment and time (i.e. survival) are additive, without any interaction. The SYSTAT package (Wilkinson 1988) was used for statistical tests.

Competitive ability of adult plants

To assess the influence of neighbouring vegetation on the gentian plants, 30 target individuals were chosen at random and the surrounding vegetation was removed by clipping at ground level to a distance of 0.3 m every two weeks during the growing season. Performance of these target plants was compared with 30 control individuals. The initial height of all individuals was measured and used as a covariable in the analysis of variance. Two types of influence of the neighbouring vegetation are considered here: (1) negative, due to suppression of growth by competition, and (2) positive, due to protec-

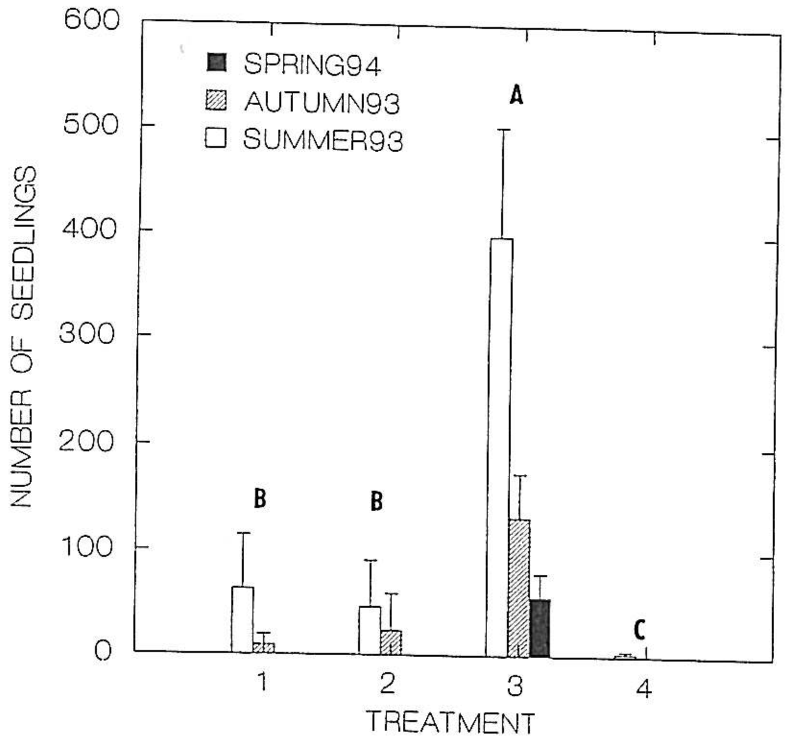

Fig. 1. Average number of seedlings in plots during various censuses. Treatments: 1 = mown; 2 = burned; 3 = cut sod; 4 = control. Error bars represent standard deviation values. Letters above bars indicate the results of the Tukey-Kramer HSD test for the summer and autumn of 1993; treatments labeled with the same letter do not differ significantly ($P > 0.05$). Identical results were obtained for both years.

Table 1. Repeated measures analysis of log-transformed counts of seedlings during the first season; the numbers of seedlings in June and September 1993 in each plot are the response variables.

Between Subjects

Source	*Ss*	*Df*	*Ms*	*F*	*P*
Block	1.532	4	0.383	1.561	0.247
Treatment	22.988	3	7.663	31.233	0.000
Error	2.944	12	0.245		

Within Subjects

Source	*Ss*	*Df*	*Ms*	*F*	*P*
Time	2.783	1	2.783	108.400	0.000
Time*Block	0.165	4	0.041	1.604	0.237
Time*Treatment	0.351	3	0.117	4.553	0.024
Error	0.308	12	0.026		

tion of individuals from grazing. Plant height and number of flowers were recorded at the end of the growing period in September 1993 and employed as the measure of plant performance. On a single day, 21 June, 1993, the photosynthetic activity of leaves of all the 60 individuals was measured in situ using a portable LCA2 analyzer.

Results

Seedling establishment

The highest germination rate was observed in plots with cut sod and the lowest in the unmown meadow. The difference between plots with burned vegetation

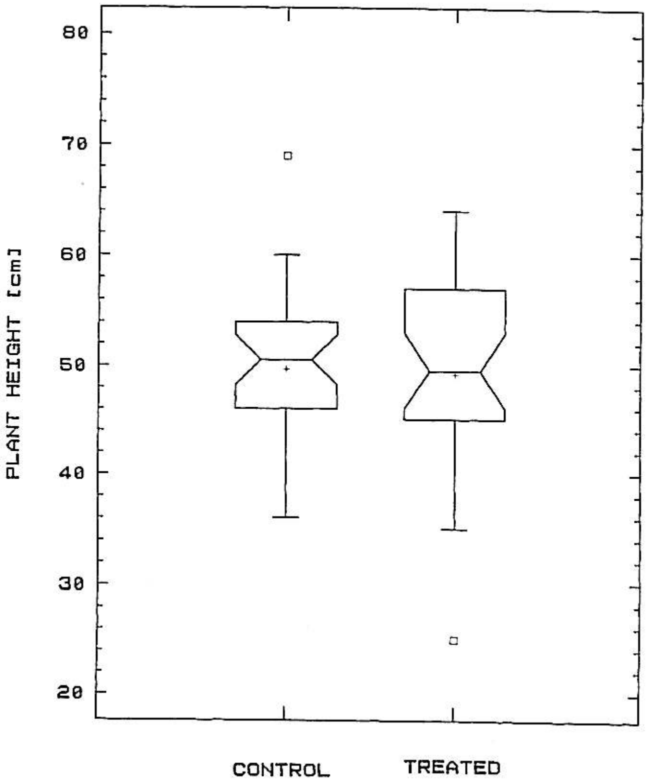

Fig. 2. Notched box and whisker plot of plant heights for control and treated plants (i.e. where the surrounding vegetation was cut). The box encloses the middle 50% of observations (i.e. between the upper and the lower quartile), with the median in the middle. The length of the notch represents an approximate 95% confidence interval for the median. Whiskers extend to the extreme points within 1.5 interquartile ranges from the quartile. Data points beyond this range are plotted individually.

and those with mown vegetation was not significant (Fig. 1, Table 1). At the end of the first growing period, the largest number of seedlings was found in plots with cut sod, whereas no seedlings were found in the control plots. The highest survival rate was observed in burned plots, 43 %, vs. 34 % in cut sod and 13 % in mown plots. Nevertheless, owing to low initial densities, absolute numbers of seedlings in plots with burned and mown vegetation were low. During the first month of the second growing period, seedlings were found only in plots in which the sod was cut and there they were surviving mainly in depressions among portions of the reversed sod.

Because individuals were found only in plots with cut sod in the second season, we only used data from the first year to test for differences in seedling germination and survival. Seedling establishment differed according to the treatment (significant between subjects Treatment, Table 1) and the same was true for the seedling survival (significant Time × Treatment interaction). Separate analyses of summer and autumn counts showed that during both periods, counts of seedlings were significantly highest in cut sod and significantly lowest in the controls (Tukey-Kramer HSD).

Competitive ability of adult plants

No differences in plant height, number of flowers per plant, or photosyntetic activity between treated and control plants were found ($P > 0.05$ for all variables (Fig. 2). Some individuals were damaged by browsing (probably deer), including both treated and control plants. Individuals which were browsed at the beginning of the vegetation period regenerated well and produced more stems and flowers than undamaged plants. Because of the low number of browsed plants the differences were not significant, neither the probability of damage by browsing nor for differences in stem and flower number between browsed and untouched plants.

Discussion

The results show that the recruitment of *Gentiana pneumonanthe* was only successful in plots where the sod was cut and turned upside down. These gaps are probably the only safe sites for the regeneration of the species. Although cutting or ploughing the sod has never been part of any conventional meadow management in Bohemia, the conditions imposed by our experimental treatment correspond approximately to those in which gaps may occur in the turf under natural disturbances, even though such natural gaps are usually smaller. Our finding is in agreement with other reports concluding that regenerating of *Gentiana pneumonanthe* occurs mainly in gaps (Oostermeijer et al. 1992a,b, 1994b; pers. observ.). Gaps were periodically created in traditionally mown meadows by horseshoe footprints, machinery tracks, missed strikes of the scythe while mowing by hand, etc. In contrast, gaps are rare or absent on abandoned meadows and in addition these meadows are covered with a thick layer or thatch of litter, which is another factor preventing seed germination. Consequently, the abandonment of meadows leads to the development of senile populations and eventually to local extinction. The remaining localities with this species should be monitored by counting the numbers of individuals at different life stages (Oostermeijer et al. 1994b). Thus, *Gentiana pneumonanthe* is an additional example of a rare species with disturbance-dependent persistence (Pavlovic 1994).

Our experiment did not demonstrate the competitive suppression of adult plants by neighbouring vegetation. However, these results cannot be generalized for other meadow types. In our oligotrophic study site, the vegetation was neither dense nor tall. In other localities where the marsh gentian has been declining the sod is usually more compact and the vegetation taller.

The results of both our experiments clearly show that the establishment phase is more critical for population persistence than later development stages, and in turn establishment is most influenced by management practice. This is probably true for many other meadow species as well, although the regeneration niche differs markedly among such species (Grubb 1977). This supports Tilman's (1993) hypothesis that the inhibition of seedling recruitment is the major cause of decreases in species diversity in productive grasslands and even though the regeneration requirements differ among species, the majority are negatively influenced by increased litter and decreased light penetration (Grime 1979; Carson & Peterson 1990). Any explanation of species richness of plant communities ignoring the mechanism of regeneration must necessarily be incomplete. Understanding the demography of particular species is of vital importance for the understanding of mechanisms that maintain species diversity.

Acknowledgements. We are indebted to Gerard Oostermeijer, Rudi Mattoni, Petr Pyšek and three referees for helpful discussions, critical comments and linguistic help and to Václav Bauer for measuring of photosynthetical activity. Research was supported by Mattoni Awards for Studies of Biodiversity and Conservation Biology (Z.K.) and the Granting Agency of the Czech Republic (grant 206/93/1179).

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Received 15 May 1995;
Revision received 22 November 1995;
Accepted 24 November 1995.

Chapter 4

Survival and viability of *Gentiana pneumonanthe* in differently managed plots over four years

Survival and viability of *Gentiana pneumonanthe* in differently managed plots over four years

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Submitted to Journal of Vegetation Sciences

Abstract. The aim of this study was to identify conditions that are essential for successful seed regeneration of marsh gentian (*Gentiana pneumonanthe* L.), a rare European species. Seedling establishment, survival and viability were studied in a four year field experiment in which greenhouse germinated seedlings were transplanted into different sized gaps: 5, 10 and 20 cm in diameter and into meadow without gaps. Gaps were situated in mown and unmown oligotrophic wet meadow, which was abandoned 14 years ago. Gentian viability was characterised by several parameters (IGVs = individuals growth values, i.e., number of leaves, stems and flowers, and plant height). Gentian mortality was high with >50% of the gentians dying in the first winter, but >25% of the surviving plants produced seeds by the end of the study. Patterns of gentian mortality differed in different sized gaps located in mown and unmown plots across years. Mowing and gap size influenced only a few IGVs. Mowing affected the number of leaves and stems only during the first year. Later, gentians differed more in shape and leaf size than in leaf number. RDAs illustrated differences between plots in which gentians survived in different sized gaps. The broad-leaved grasses together with clonal *Prunella vulgaris* and hemiparasitic *Pedicularis sylvatica* occurred in plots with high gentian mortality. This study confirmed that gaps are essential for the successful regeneration of *Gentiana pneumonanthe* in oligotrophic wet meadows. Although larger gaps are characterised by higher initial mortality rates caused by harsh microclimatic condition, they are necessary in long-time abandoned meadows. Regularly created small gaps are sufficient for gentian establishment only in traditionally managed meadows.

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for ...
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Keywords: Gaps; Establishment; Endangered species; Oligotrophic meadow; Management.

Nomenclature: Rothmaler (1976); Váňa (1997) for bryophytes.

Introduction

It is well known that the creation of gaps within plant communities is often a key requirement for establishment of plants from seeds. This is equally true for woodlands (Brown & Archer 1988) and grasslands (van der Maarel & Sykes 1993; Bullock et al. 1995; Špačková et al. 1998; Kotorová & Lepš, 1999). In natural habitats, the gaps are created by many mechanisms, such as the death of perennials as result of environmental conditions or disease, the activities of grazing or burrowing animals or erosion. Gaps provide regeneration niches for species present in communities as well as for migrants (Pakeman et al. 1998). In traditionally managed meadows and pastures in Central Europe gaps were created not only by natural mechanisms but also periodically by horses, cattle, sheep, goats, machinery tracks, and by missed strikes of the scythe while mowing. Grazing animals also dispersed seeds in hoofs and hair.

Recently, traditional management practices have been considered uneconomical, and meadow management is either intensified, or the meadows are abandoned. One of the most rapidly vanishing grassland communities of Central Europe are oligotrophic species-rich meadows. Their species richness is very high; at the scale of metres they belong to the world's most diverse communities (Kull & Zobel 1991). Large areas of wet oligotrophic meadows were drained and/or fertilised and thereby changed to species-poor meadows (often monocultures) during the second half of the 20th century. Many typical species of these meadows became rare and a number of them are endangered. Formerly common plant species, or „new rare" (sensu Huenneke 1991), are relegated to small populations as a result of the fragmentation, destruction and habitat deterioration. These small and fragmented populations can only survive if certain ecological and genetic conditions are met. A better understanding of life strategies of the endangered species and of the relationships between the plant populations and the vegetation in which they are a part are essential tools for suitable management and conservation of species (Synge 1985).

In most European countries these trends can be exemplified with Gentianaceae species. Both the number of populations and population size of many *Gentiana* and *Gentianella* species rapidly decreased during the second half of the 20th century (details in Oostermeijer et al.1992, Lennartsson & Svensson 1996, Stőcklin & Fischer 1999). Several species died out and others survive only in few and small populations. Population biology and genetics of many *Gentiana* and *Gentianella* species have been studied in several European countries to answer questions about reproductive strategies, competitive ability, viability and genetic structure of populations. Better knowledge about demography (Fischer & Matthies 1998 a,b), reproduction (Petanidou et al. 1995 a,b, 1998; Luijten et al. 1998, 1999; Lennartsson 1997; Milberg 1994), genetics (Fischer & Matthies 1998c) will help to protect the surviving populations and optimise the management practices in areas where gentians occur. One of the most studied *Gentiana* species is the marsh gentian, *Gentiana pneumonanthe*. Several authors (Oostermeijer et al. 1994b, Rose et al. 1998, Kesel & Urban 1999) have studied germination of G. pneumonanthe. All of them report that gaps and low competition microsites are essential for successful germination and seedling recruitment of the marsh gentian. Most of these studies were done in *Erica tetralix* dominated acid heathlands, which have lower species richness than wet oligotrophic meadows. The aim of this study was to define which gap size and which type of meadow management are the best for successful recruitment of marsh gentian seedlings.

ital.

Methods

Study site and study species

This study was conducted in a 2.5 ha oligotrophic species-rich meadow located 10 km southeast of České Budějovice, Czech Republic, 48°57′ N, 14°36′ E, 510 m a.s.l. According to records from the nearest meteorological station (Lhenice, 5 km SE) mean annual precipitation is 665 mm, mean annual temperature is 7°C, and mean annual number of days with continuos snow cover is 57 (unpublished data, the Czech Hydrometeorological Institute in České Budějovice). The vegetation can be described as a *Molinion* association with some species indicating a transition to a *Violion caninae* association. For a detailed description see Špačková et al. (1998).

belongs to
alliance

This previously regularly mown traditionally managed meadow was abandoned 14 years ago. Vegetation development after meadow abandonment is influenced by several factors, such as the hydrological regime (timing and duration of hydric conditions), time of abandonment, diaspore resources (near forest and field), etc. and can be characterised as a sequence of successional stages. Several endangered or rare species, e.g., *Pedicularis sylvatica, Dactylorhiza majalis* and G. *pneumonanthe* occur here. The *G. pneumonanthe* population is senile (sensu Oostermeijer 1994b; no seedlings or juvenile plants were found) and the population size is very low (<10 individuals). Two larger populations (30 and 500 flowering gentians) occur less than 1,5 km from the study site.

and

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Gentiana pneumonanthe, a Euroasian element, is a typical species of wet oligotrophic meadows dominated by *Molinia caerulea* (Oberdorfer 1962). In the Atlantic part of Europe, G. *pneumonanthe* occurs in wet acidic heathlands and on the borders of peat bogs (Simmonds 1946, Chapman et all. 1989, Oostermeijer et all. 1994a,b; Oostermeijer et all. 1995). *Gentiana pneumonanthe* is a perennial species that lives several decades and reproduces only by seed. Based on demographic studies six different ontogenetic stages in marsh gentian populations have been distinguished: seeds, seedlings, juveniles, vegetative adults, generative adults, and dormant (Oostrermeijer at al. 1994b). The normal developmental sequence of the individual is from seed to generative adult. Seedlings and juveniles stages usually each last one year but there is variation in transition times from vegetative to generative adult. Protandrous flowers produce high numbers of small and light seeds; about 300 – 700 per capsule (Simmonds 1946). Seeds can germinate instantaneously after ripening in autumn as well as after cold stratification in spring (Thompson 1969).

Experimental design

collected n. growing

Seeds of *G. pneumonanthe* were collected from plants growing in the study site in autumn 1995 and stored under cool dry conditions. The experiment was established in April 1996 when 7 week old greenhouse grown seedlings were transplanted into 5, 10 and 20 cm diameter gaps and into meadow without gaps. Gaps were situated in the middle of 1,5 x 1,5 m homogenous plots (n=40). Twenty of the plots were mown annually in June and twenty were unmanaged. Mown biomass was carefully gathered and removed from plots. The experiment is a split-plot design, with mowing being the main-plot factor and gap size the within-plot factor.

jak určili ?
jak uspořádány ?
jak vybrány ?
= variable ?

During the first two years of the experiment individual growth values (IGVs) of all surviving individuals were measured twice a year (in June and September). I used IGVs as a general term for the number of stems, leaves, flowers and height of plants. Number of leaves and seedling diameters were recorded in 1996 and number of leaves and stems per plant and plant height were measured in 1997. In 1998 and 1999 these same IGVs plus the number of flowers per plant were recorded and only in September. Only one measurement during the vegetative season was sufficient because no differences in IGV values were found between June and September.

jak měřeno ?
= leaf ?
in aug ?

In September 1999, at the end of experiment, vegetation structure, living biomass, and biomass of litter and moss in each of the 40 plots were determined. Percent covers of all vascular species, moss layer and litter were visually estimated in the central 1 x 1 m plot. Vegetation was clipped in the central 0,3 x 0,3 m plot. The clipped biomass together with moss and litter was raked up, saved in plastic bags and transported to the laboratory. The living biomass, litter, and moss were carefully sorted, oven-dried (70°C) and weighed.

was

do jakých hodin ?

Data analysis

odpovídá tomu použitý ANOVA?

Data are in the form of repeated measures. IGVs of all surviving individuals were determined six times. Corresponding survival analyses were used to test the effect of gap size and plot management type on survival of young *G. pneumonanthe* individuals.

IGVs were log-transformed – using log (x) to achieve homogeneity of variance. Effect of mowing and gap size on IGVs of young gentians was analysed by nested design ANOVA-models. Nested design ANOVA models, which are part of the General Linear Models (GLMs) in the STATISTICA 7.0 package, made it possible to analyse an incomplete data matrix of the split-plot design, which was caused by the mortality of young gentians. MANOVA-models were used to test the effects of gap size and mowing on several IGVs simultaneously in each period of the experiment.

dekadický ? (log(10))

Logistic regression was used to test the effect of the number of leaves per plant, as a degree of vitality, on survival of individuals to the next year. Corresponding repeated-measures ANOVA-models were used only for individuals that survived all four seasons of the experiment.

For species composition, Redundancy Analysis (RDA) in the CANOCO package (ter Braak 1990), was used to test the effect of mowing on species composition and correlation between species composition and survival of young gentians in different sized gaps. RDA, a method based on a linear species response, was used because species composition in the plots was rather homogenous and explanatory variables were categorical. Data were standardised by species because there were only several species with high percent cover and most species occurred in low abundance. Because only planted individuals of *G. pneumonanthe* occurred in plots, the species was made passive in the analysis so as not to influence ordination results. By using the survival of gentians as an explanatory variable, the „reverse" analysis was performed (*i.e.*, species composition might influence the survival, not vice verse). In this way differences between plots where gentians survived and plots where they did not were tested.

few

a

occur?

In all cases, the significance of results was tested by the Monte Carlo permutation test. Programs CanoDraw and CanoPost (Šmilauer 1992, ter Braak & Šmilauer 1998) were used for graphical presentations of ordination results.

Logistic regression models in the STATISTICA package (Anonymus 1999) were used to test the effect of the cover of all vascular species, cover of moss layer, living biomass, litter biomass and moss biomass on gentian survival.

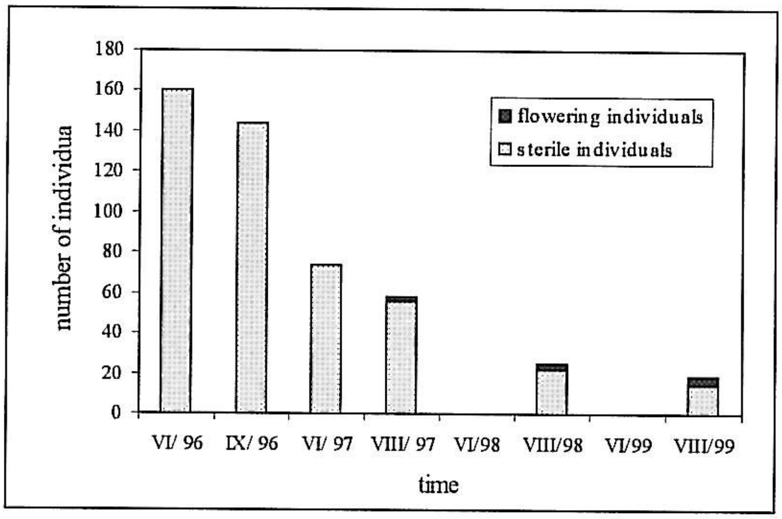

Fig. 1. Total number of surviving individuals of *Gentiana pneumonanthe* and proportion of flowering individuals during four years experiment.

Results

zvolení

Survival of individuals

proč je hodnoceno tolik místa N.S. - vysvětlení ?

Marsh gentian mortality over the four years of the experiment was high. From 160 individuals planted in spring 1996 only 19 gentians (~12%) survived till September 1999. Two individuals flowered in the second vegetative season and more than 25% of the surviving individuals flowered in summer 1999 (Fig.1). Mortality rates of young gentians in different sized gaps in mown and unmown plots differed across years (Fig. 2). During the first vegetative season more seedlings died in 20 cm diameter gaps located in mown plots than in gaps of the same size located in unmown plots (Fig.2a). There were no differences in number of surviving individuals in smaller gaps between mown and unmown plots (Fig. 2c). In all of the gap types, >50% of the gentians died during the first winter. In summer 1997, mortality was very low and there were no significant differences in the number of surviving individuals between gaps located in mown and unmown plots. Young plants from mown and unmown plots differed in their IGVs (see below). Mortality rates decreased during the winters of 1997/98 and 1998/99, with more individuals dying in unmown plots (Fig.2). There were no significant differences between mown and unmown plots (12,5% and 11,25%). Survival analysis showed no significant influence of gap size and plot management on gentian survival during four years.

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testováno!

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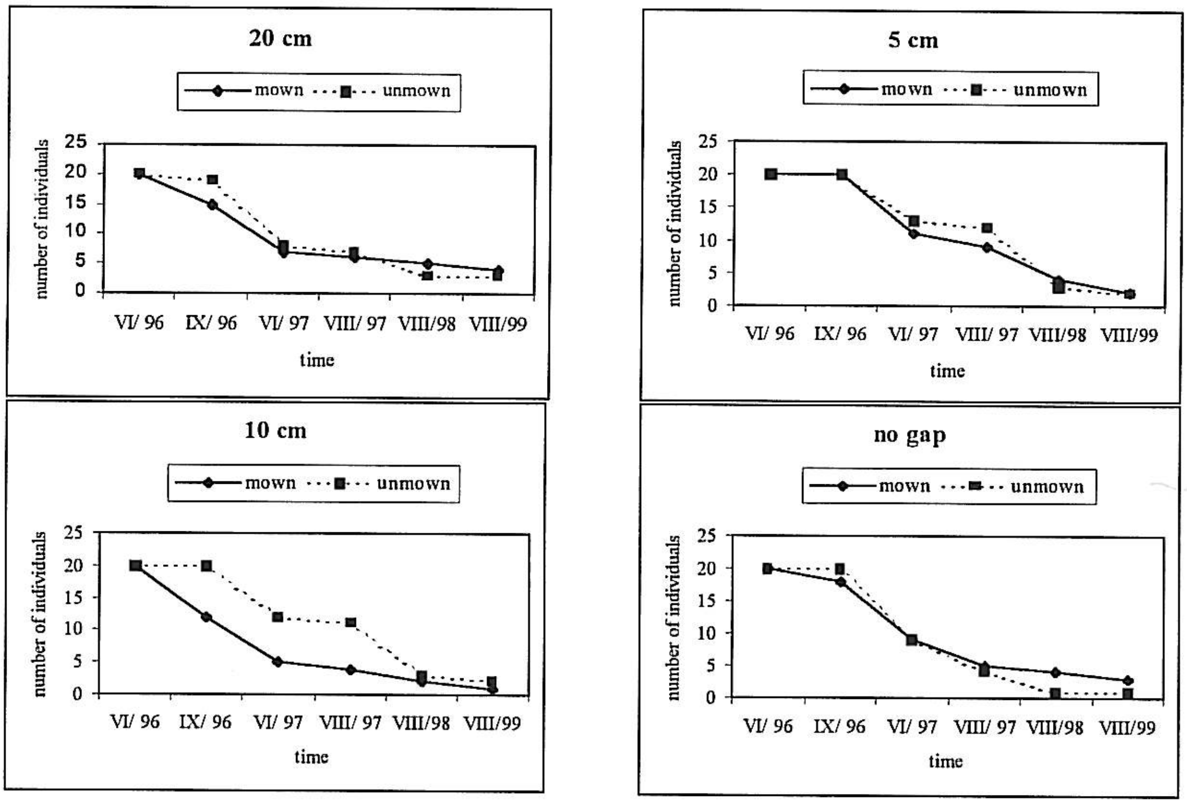

proč ne směrodatné SE ?

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Fig. 2. Numbers of individuals of *Gentiana pneumonanthe* surviving in different size gaps during four years experiment.

Individual growth values

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Few IGVs were affected by mowing (Table1). Gap size and gap size-mowing interactions affected no IGVs. Mowing significantly affected the number of leaves and stems only in 1997. Individuals surviving in mown plots had more leaves and stems than in unmown plots. Similar trends were observed in 1998 and 1999.

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Table 1. The effect of gap size, mowing, and gap and mowing interaction on the number of leaves and number of stems per plant, and height of plant during for years experiment. The results of split-plot ANOVA analyses and MANOVA analyses are shown. The significance of F-value are: p< 0.05 are in bold. Leaf numbers were also effected by gap-mowing interaction in September 1996 and stem numbers were effected by gaps in June and August 1997 but differences are out of significant level p<0.05. P> 0.05 → N.S. ?

	September 1996		June 1997		August 1997		August 1998		August 1999	
Number of surviving individuals	144		74		58		25		19	
	F	p	F	p	F	p	F	p	F	p
Number of leaves per plant										
plot	2.01	**0.003**	0.48	0.976	1.14	0.350	35.82	0.130		
mowing	0.64	0.427	7.48	**0.009**	0.02	0.880	0.03	0.857	1.41	0.270
gap	1.39	0.250	1.15	0.343	1.20	0.325	3.03	0.394		
gap-mowing interaction	2.28	0.083	0.18	0.909	0.49	0.694	3.48	0.371		
Number of stems per plant										
plot			0.68	0.857	0.95	0.553	0.96	0.675		
mowing			4.25	**0.045**	0.37	0.548	0.91	0.372	0.80	0.397
gap			2.30	0.092	2.39	0.085	0.12	0.936		
gap-mowing interaction			0.27	0.847	0.09	0.964	0.12	0.936		
Height of plant										
plot			1.11	0.379	1.75	0.057	13.40	0.212		
mowing			1.82	0.185	0.27	0.603	1.29	0.274	0.97	0.353
gap			1.10	0.362	1.02	0.396	3.84	0.355		
gap-mowing interaction			0.27	0.850	1.41	0.257	4.68	0.324		
MANOVA										
plot	1.21	0.221	0.95	0.545	1.17	0.323	35.82	0.130		
mowing	0.04	0.843	0.82	0.370	0.09	0.766	0.03	0.857	1.41	0.270
gap	0.58	0.630	0.94	0.431	1.20	0.325	3.03	0.394		
gap-mowing interaction	1.38	0.252	1.26	0.301	0.49	0.694	3.48	0.371		

MANOVA models were used to analyse all available IGVs in each period of the experiment. Neither gap size, mowing, nor gap size-mowing interactions influenced IGVs. Logistic regression showed that there is no correlation between the number of leaves per plant and the survival of individuals to the next year.

Only data for gentians surviving over for seasons (1996 – 1999) were analysed by repeated measures ANOVA. Data from four years were available for the number of leaves per plant (the numbers of leaves were recorded six times). Data from three years were available for the number of stems and plant height because these parameters were recorded since the second year of experiment. Repeated measures ANOVA found a significant effect of gap size-mowing interaction on the number of leaves per plant ($p<0.025$) (Table 2). Number of leaves were also influenced by mowing ($p<0.052$) NS and gap size ($p<0.061$) NS. Mowing-time interaction affected plant height ($p<0.028$). Differences in plant height of gentians in different sized gaps located in mown and mown plots are shown in Fig. 3. The number of stems per plant was affected by the size of gaps ($P<0.022$).

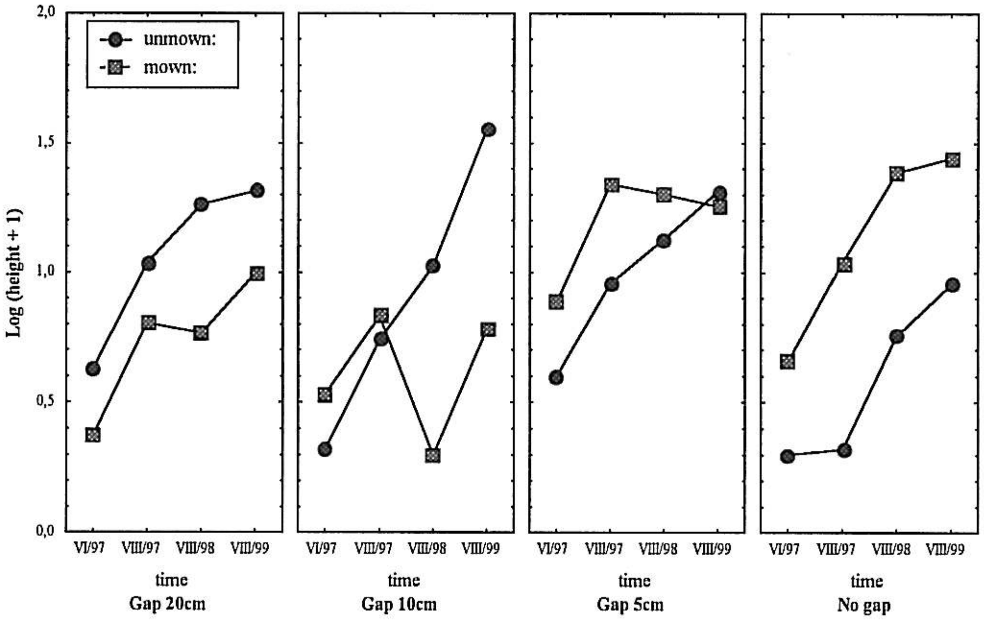

Fig. 3. Log- transformed heights of plants [Log (x+1)] surviving in gaps of different size located in mown and unmown plots. The heights were recorded since the second year of the four years experiment.

Species composition and biomass

Vegetation structure of mown and unmown plots significantly differed (Table3; $P>0.002$). RDA analysis showed that *Molinia caerulea, Carex panicea, Juncus conglomeratus, Equisetum palustre, Scorsonera humilis, Lysimachia vulgaris*, and *Ranunculus nemorosus* occurred predominantly in unmown plots (Fig. 4). The clonal *Prunella vulgaris* and *Veronica officinalis* together with *Pedicularis sylvatica, Ranunculus acris* and *Cirsium palustre* were more abundant in mown plots.

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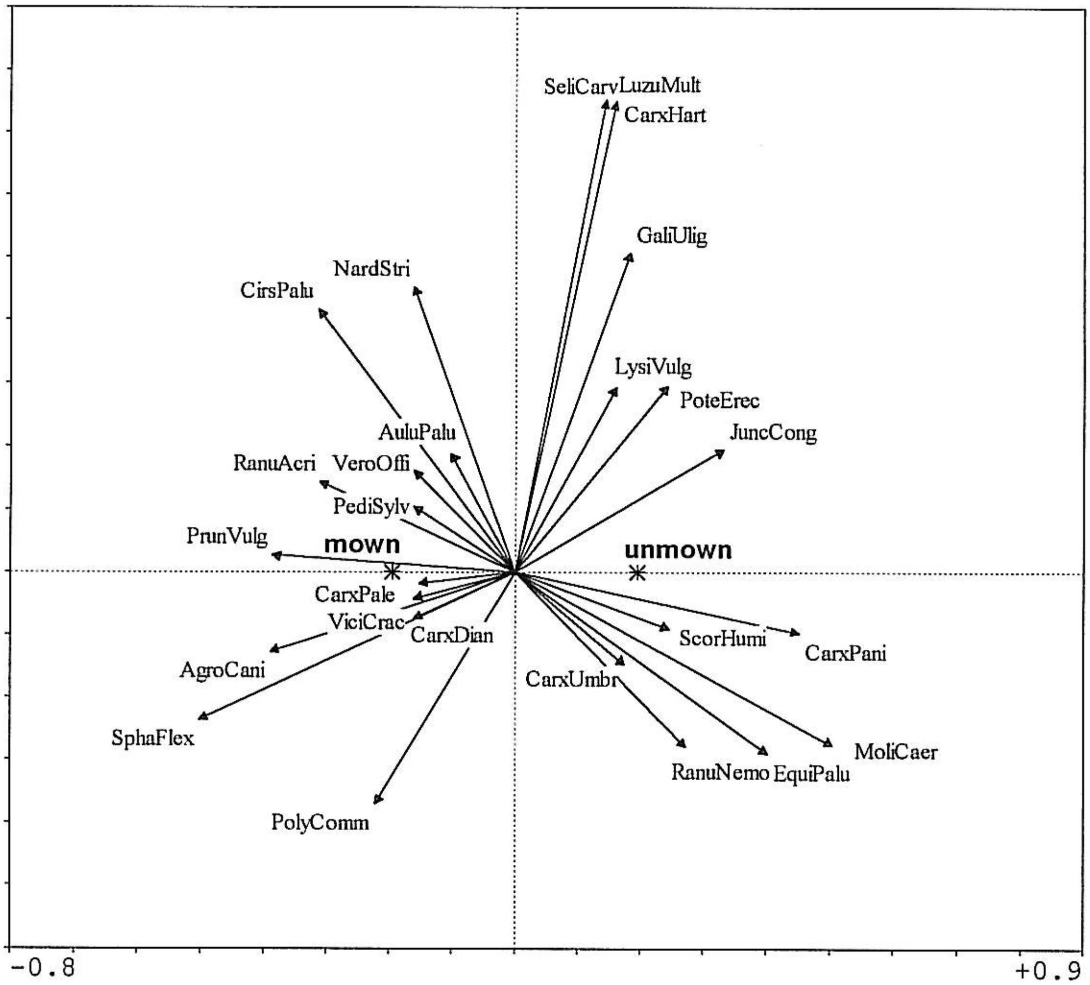


species composition

Fig. 4. Results of the RDA analysis of vegetation structure in mown and unmown plots. The horizontal and vertical axes are the first and the second RDA axes respectively. The first axis is highly significant (P<0.002). Abbreviations of the species names are in Appendix 1.

Table 2. Repeated measures analysis of log-transformed numbers of leaves, numbers of stems and height of plants; data only for individuals surviving over for seasons (1996 – 1999) were analysed. The significance of F-value are: $p < 0.05$ are bold. Leaf numbers were significantly effected by gap-mowing interaction and also separately by mowing and gap size, differences were closed to significant level.

/ ca

	Leaves		Stems		Height	
	F	p	F	P	F	P
mowing	5.20	0.052	2.46	0.156	0.00	0.956
gap	3.73	0.061	5.65	**0.022**	1.18	0.377
time	15.75	**0.000**	3.48	**0.031**	18.41	**0.000**
gap-mowing interaction	5.43	**0.025**	2.88	0.103	2.70	0.116
mowing-time interaction	0.49	0.783	0.68	0.574	3.60	**0.028**
gap-time interaction	0.85	0.621	1.53	0.194	1.52	0.196
mowing-gap-time interaction	1.50	0.151	0.18	0.994	1.56	0.186

proč ne ± ? (další tab. 1)

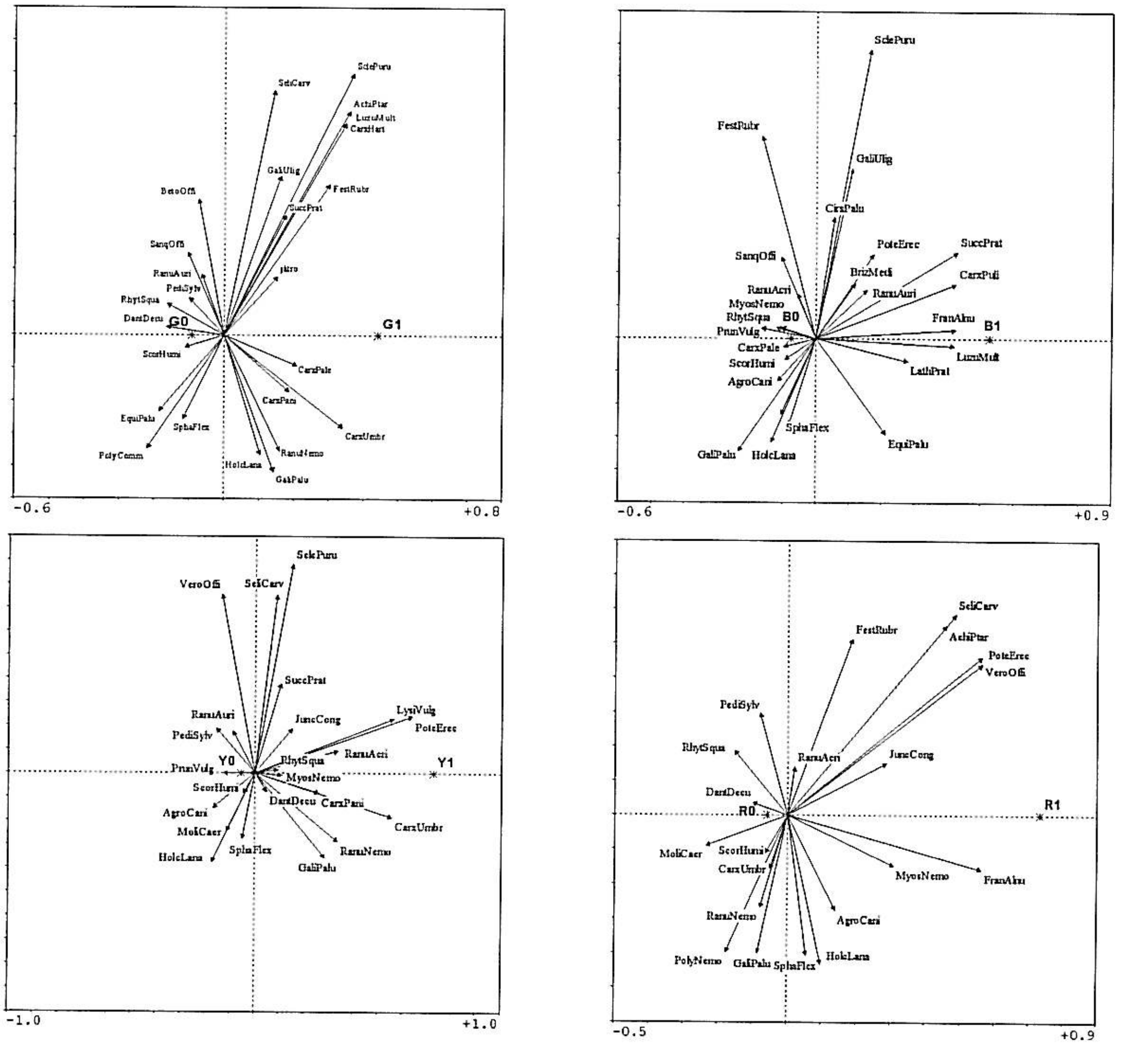
Table 3. Characteristics of the RDA analyses of vegetation structure in mown and unmown plots. [a] – Variability in species data explained by the first axis (%); [b] - F-ratio on the first RDA axis; [c] - Significance of the first RDA axis estimated using Monte Carlo permutation test; [d] - Total variability in species data explained by the first four axes (%).

	1-st axis variability [a]	F[b]	P[c]
Mowing	4.9	1.972	**0.002**
Without gap	5.3	2.12	**0.022**
5 cm gap	4.2	1.646	0.056
10 cm gap	5.3	2.12	**0.022**
20 cm gap	3	1.178	0.184
	1-st 4 ax. variab.[d]	F[b]	P[c]
All gaps	17.3	1.836	**0.002**

Also, mosses occurred mostly in mown plots. Surrounding vegetation affected *G. pneumonanthe* survival in different sized gaps (Table 3). RDA analyses show the differences between plots in which gentians survived in different sized gaps (Fig. 5). In the plots with high gentian mortality in large gaps mainly broad-leaved graminoids, *Molinia caerulea, Holcus lanatus, Agrostis canina, Festuca rubra, Prunella vulgaris*, and *Pedicularis sylvatica* occurred. Survival of young gentians in

[illegible]

small gaps and in plots without gaps was correlated with occurrence of *Luzula multiflora, Succisa pratensis,* and *Potentila erecta* (Fig.5c, 5d). In plots with 5 cm diameter gaps and without gaps,



[illegible]

Fig. 5. Results of the RDA analysis of vegetation structure in mown and unmown plots separately for gentians growing in gaps of different size. G0 = gaps with 20 cm in diameter located in unmown plots; G1 = gaps with 20 cm in diameter located in mown plots; Y0 = gaps with 10 cm in diameter located in unmown plots; Y1 = gaps with 10 cm in diameter located in mown plots; BO = gaps with 5 cm in diameter located in unmown plots; B1= gaps with 5 cm in diameter located in mown plots; R0 = gentians growing in unmown plots without gaps; R1 = gentians growing in mown plots without gaps. The horizontal and vertical axes are the first and the second RDA axes respectively. By using the survival of gentians as explanatory variable, the „reverse" analysis was performed (in fact the species composition might influence the survival not vice verse). Abbreviations of the species names are in Appendix 1.

[illegible]
[illegible]
[illegible] ?

in which gentians survived, seedlings of *Frangula alnus* occurred. Seedlings of *Selinum carvifolia, Myosotis nemorosa, Lysimachia vulgaris, Lychnis flos-cuculi, Ranunculus acris, Ranunculus auricomus, Galium uliginosum, Galium palustre* and several grasses and sedges occurred in gaps of all sizes. Seedlings of *Selinum carvifolia, Lysimachia vulgaris, Ranunculus acris, Achillea ptarmica,* and *Agrostis canina* did well in gaps and suppressed young gentians. Lower cover of broad-leaves graminoids and other dominant species facilitated *Frangula* seedling establishment as well as gentian survival. Spreading rhizomes from the surrounding vegetation decreased gap area over the four years. About 20% of the 10 and 20 cm gaps were overgrown by clonal *Prunella vulgaris, Veronica officinalis,* and the hemiparasitic *Pedicularis sylvatica*. Also, *Sphagnum flexuosum* overgrew gaps of all sizes but gentians usually survived in these gaps in contrast to gaps overgrown by clonal dicots.

spekulace

spekulace

Logistic regression models, independent of gap size, showed that vascular plant and moss cover and moss, litter and living biomass did not affect gentian survival (Table 4). A negative relationship between vascular plant cover and gentian survival (p=0.018) was found only for individuals growing in plots without gaps. Moss cover and biomass were positively correlated with gentian survival in plots without gaps (p=0.006, p=0.049 respectively). This correlation is partially an artefact because high moss cover and biomass were typical of plots with lower broad-leaved grass abundance.

zde třeba opravit tabulku

Proč ne [illegible] P= P? jak [illegible]

problém interpretace nikoli korelace samotné

dobře nic nevyšlo?

Co je v tabulce? Přežívání G. p.?

Tab. 4. Logistic regression analysis of gentians survived in gaps of different size in dependence on the % cover of all vascular plants, % cover of mosses, the biomass of mosses, litter and living biomass in autumn 1999.

		Cover E_1	Cover E_0	Biomass of mosses	Biomass of litter	Living biomass
All gaps	Chi-square	0.00	1.48	2.03	0.08	0.09
	P	0.954	0.224	0.155	0.781	0.761
Without gap	Chi-square	5.63	7.51	3.88	1.33	0.50
	P	**0.018**	**0.006**	**0.049**	0.066	0.480
5 cm gaps	Chi-square	0.15	0.19	0.13	0.73	0.67
	P	0.696	0.665	0.721	0.394	0.415
10 cm gaps	Chi-square	1.37	0.01	0.16	0.00	0.39
	P	0.242	0.926	0.693	0.226	0.988
20 cm gaps	Chi-square	1.08	0.08	0.07	0.00	0.00
	P	0.298	0.777	0.796	0.946	0.983

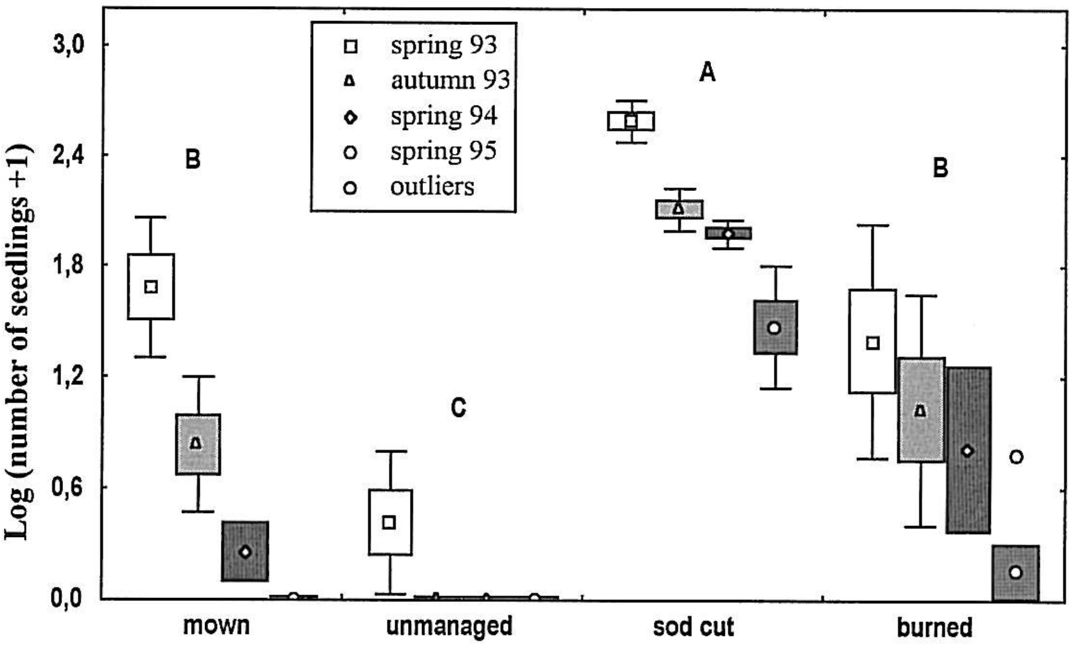
srovnávací výsledky jak měřeno?

ale výsledky ukazují, že neovlivnily, ne?

Discussion

The results of this experiment show that gaps of different sizes and meadow management can effect the survival and viability of *G. pneumonanthe*, principally in the initial life stages. This corresponds to other studies that have found gap size and shape to influence colonisation and establishment in gaps (Goldberg & Werner, 1983; Kotanen 1997). The intensity of this influence differs across life stages. *Gentiana pneumonanthe* is a typical example of a rare species with disturbance-dependent persistence (Pavlovic 1994), but the intensity and scale of these disturbances are still unclear. Adult individuals of *G. pneumonanthe*, which can live several decades, produce large quantities of seeds but most of them are lost. Several authors report that seeds germinate successfully only in sites with open bare soil (Oostermeijer et al. 1994b, Křenová & Lepš 1996, Kesel & Urban 1999). In suitable sites a germination rate of 20-25% is followed by high seedling mortality. High gentian mortality was observed both in this experiment, with seedlings of *G. pneumonanthe* planted in gaps of different sizes, and in our previous experiment in which the germination and survival of *G. pneumonanthe* seedlings were tested (Křenová & Lepš 1996). More than 60% of the seedlings died over the first summer in plots with cut sod, the treatment with the highest germination, and >85% of them died in mown plots with small gaps occurred (see Fig. 6).

patří do výsledků, [illegible] [illegible] Fig. 1



?

Fig. 6. Average number of seedlings in plots under various censuses. Geometric figures represent means, boxes represent standard errors, and whiskers represent standard deviation. Letter above boxes indicate results of the Tukey-Kramer HSD test for the summer 1993; treatments with the same letters do not differ significantly (P>0.05).

Proč je uveden test pro data, pro které [illegible] [illegible] hodnoty?

In comparison to extremely high seedling mortality under natural condition over the first year the mortality rate of transplanted greenhouse germinated gentians was much lower. Jiffy pots in which seedlings where transplanted could protect them in the initial life stages. The pots probably reduced competition intensity and various stress factors. The protective role of Jiffy pots decrease with time.

[illegible] obr. 2 [illegible] 12

Also, the self-thinning effect was eliminated in this experiment. Several factors may have caused the higher seedling mortality observed in gaps in mown plots than in unmown plots over the two first years (Fig. 2). Microclimatic conditions such as high temperatures, drought or flooding in extremely deep gaps together with herbivores (insects, slugs etc.) may stress gentian seedlings. Over-heating in 20 and 10 cm diameter gaps probably caused the higher seedling mortality in mown plots due to the lack of shading by surrounding vegetation. Also, many seedlings may die from drought in gaps located in mown plots over the first weeks. Because vegetation was closer and the litter layer accumulated faster the mortality rate increased over time in unmown plots. As could be predicted, over four years a higher proportion of gentians died in small gaps and in plots without gaps than in the 10 and 20 cm diameter gaps, as a consequence of higher light and less competition for space. Almost all of the individuals planted in large gaps, which survived the first year, did well and flowered the following seasons. On the other hand, a higher proportion of gentians died off in small gaps and plots without gaps, where the mortality rate was less in the initial period. These results show that established individuals develop well in larger gaps for many years and reproduce. But individuals growing in sites with dense vegetation cover and high litter accumulation remain stressed and their viability decreases and many die. The high mortality rate in the initial stages of the life cycle corresponds to long-time demographic monitoring of *G. pneumonanthe* populations (Rose et al. 1998), where >20% mortality rate during the first year was recorded for gentian populations growing in south England heathlands.

nesignif!

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In contrast to my expectations, no differences in leaf number between plants growing in different sized gaps were found (Table1). Only stem number showed a response to gap size, and this was not significant at P=0.05. From the ANOVA it can be concluded that gap size does not significantly effect individual growth values, an index of young gentian viability. The number of leaves per plant was supposed to be used as a viability index but young gentians differ in leaf size more than in leaf number. Individuals grown in unmown plots created thin leaves that were longer and wider than leaves of plants in mown plots. They also differ in colour shaded plants in unmown plots often having yellow or yellow-green leaves. Although it wasn't measured photosynthetic activity was probably lower for gentians in unmown plots than in mown plots. Also, mowing affected the number of stems and leaves per plant only in initial stages of the life cycle. Later in the experiment gentians died independent of their individual growth vales. Different results were found by repeated measures which was calculated only for individuals surviving over all four seasons of the study (Table 2). Stem number was affected by gap size and leaf number was affected by the gap size-mowing interaction. Mowing of plots could influence survival and viability of young gentians also negatively because some individuals could be cut off by the mowing process or eaten by deer in mown plots. Some of them regenerated from basal buds and have more stems. Mowing also influenced microclimatic conditions (light, temperature and humidity conditions differed between mown and unmown plots; Lepš 1999).

nejsou v úvodě uvedeny

jaký je rozdíl mezi žádným a NS rozdílem?

mělo by být v úvodu (nebo v úvodě)

úzko? kde jsou výsledky

měřil.

y

The results of this study show that mortality in initial stages was much more influenced by abiotic conditions in gaps than in later stages of the gentian life cycle when competition with surrounding vegetation and other species colonising gaps was much more important. Competition with surrounding vegetation had a stronger effect on gentians growing in small gaps and in plots without gaps. More competitors colonised small gaps than large gaps because small gaps are acceptable for species of variant life strategy (Bullock et al. 1995). Mowing also affected gentian survival indirectly through its influence on vegetation structure. *Molinia caerulea, Carex panicea, Scorsonera humilis*, and *Lysimachia vulgaris* (Fig. 4), which are typical of abandoned oligotrophic wet meadows, dominate in unmown plots. Their high cover and broad leaves shade seedlings and create a dense litter layer that cause very high mortality in young gentians planted in unmown plots with small gaps or without gaps. In mown plots where successional changes were blocked, several heliophytes, such as *Cirsium palustre, Myosotis nemorosa, Prunella vulgaris* and *Pedicularis sylvatica* occurred. Also mosses profited from better light conditions in mown plots. High moss cover was correlated with high mortality rates during the first years of experiment. Both protective (Ryser 1990, During & van Tooren 1999) and inhibitive (Keizer et al. 1985; Špačková et al.1998) roles of mosses for seedling establishment in grassland communities have been reported. The protective role is probably more important in dry grasslands where mosses may protect seedlings from over-drying or predation (van Tooren 1988). In wet meadows, the inhibitive role is probably more important (Malmer et al. 1994; Svensson 1995). Both Rabotnov (1987) and Špačková et al. (1998) reported positive effects of moss removal on germination and early survival of seedlings, however, later survival was better where mosses were not removed. These results correspond with results of this study where high gentian mortality was correlated with *Sphagnum* species over-growing gaps during the first two years. Larger gentians can survive in gaps that are overgrown by *Sphagnum* and benefit from *Sphagnum* protection.

survival anal.
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nelze oddělit
od ost. fakt.

multiplikativní

Many species in perennial grasslands are capable of reproducing by both seeds and clonal growth. The different ability of different species to colonise gaps by these two modes can be one of the most important variables affecting how gaps influence species composition (Bullock et al. 1995). Stronger competitive effects were observed between young gentians and clonal *Prunella vulgaris* and *Veronica officinalis* and to a lesser extent *Pedicularis sylvatica*. *Prunella vulgaris* completely overgrew several 5 and 10 cm gaps over two years. Likewise, *Pedicularis sylvatica* seedlings grew to completely cover several 5 and 10 cm gaps although the nearest individuals of this rare species were several meters away from plots. It is possible that ants could move seeds of this species. Young gentians survive in gaps overgrown by *Pedicularis* longer than in gaps overgrown by *Prunella*. Seedlings of several common species were also found in gaps, the most common were *Cirsium palustris, Achillea ptarmica, Ranunculus acris, Lysimachia vulgaris, Agrostis cannina,* and *Galium uliginosum.*

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~ 1 odst.

The relative contributions of seedling establishment and clonal growth to gap colonisation have been little studied in grassland. Rapp and Rabinowitz (1985) reported that excluding clonal colonisation of artifical gaps in prairie did not effect seedling colonisation of the gaps. They hypothesised that there were only weak interactions between these two types of colonists. It seems that interspecific competition plays a more important role in the later life cycle stages when both seedlings and clonal ramets create more biomass and they compete with each other for space as well as for resources. Over the course of the study gap size decreased owing to expansion by surrounding vegetation and the accumulation of litter. In harmony with basic ecological theory life strategies, growth rate, and the time and intensity of colonisation of gaps affected competition intensity and the ability of young gentians to survive in gaps. Young gentians growing in gaps that were not colonised by other species until the second year or later grew well. Colonists were only small individuals or ramets during the first years of their life. Well-established individuals of *G. pneumonanthe* can survive in highly competitive environments for many years. But for the successful establishment of *G. pneumonanthe* seedlings strong disturbances that decrease competition appear to be crucial. This corresponds with the occurrence of invasive populations of marsh gentian (population with high proportion of seedlings and juvenile plants) in communities of initial successional seres (Oostermeijer 1994b, Rose et al. 1998). In *Erica tetralix* - heathlands of Western Europe, the „invasive" populations of *G. pneumonanthe* occurred mainly in sites where vegetation cover and soil surface had been disturbed (Oostermeijer et all. 1994b). Large sod cutting together with periodic burning was one of the typical management practices in heathlands areas. Moderate to occasional cattle grazing (Wittig 1996) or burning (Chapman et al. 1989) applied in short intervals (4-8 years) are recommended to managers of *G. pneumonanthe* locations. Kesel & Urban (1999) found that small-scale gaps and brief inundation or moist soils in gaps promoted the germination and establishment of *G. pneumonanthe*. In their interpretation, small-scale gaps varied from 0.25 to 4 m^2 in size and large scale cut plots were 1500 m^2. This scaling corresponds with the spatial structure of heathlands where bare soil can reach up to 50%, depending on the plant community and the successional sere (Oostermeijer et al. 1994b). The spatial structure of Central European wet meadows differs from heathlands. Only small-scale gaps (several cm^2) are created by missed scythe strokes while hand-mowing in traditionally managed meadows or by hooves of horses, sheep, cattle or goats in pastures. Only machinery and the gathering of domestic and wild animals could create larger and more intensive disturbances. Large-scale disturbances (several square metres) can be very important for the survival of *G. pneumonanthe* in recent times when many populations occur in abandoned meadows. This management is typical of military training areas where the vegetation cover is trampled by sporadic army activities. Populations of *G. pneumonanthe* take advantage of these locations, seeds germinate in open places, population structure is rejuvenated and the populations can survive several decades. Large-scale and intensive disturbance management seems to be useful for long-time abandoned meadows where a thick litter layer together with a dense canopy can block the restoration ability of traditional management practices such as mowing or moderate grazing.

The results of this study confirmed that gaps or sites with bare soil are necessary for germination and survival of seedlings of *Gentiana pneumonanthe*, an endangered species of the European flora. Gap creation intensity and scale is dependent on the spatial density and composition of the surrounding vegetation. Although higher initial mortality rates caused by harsh microclimatic conditions are typical for large gaps, the large gaps (several dm^2) are necessary for successful seedling establishment in long abandoned meadows. Juvenile gentians growing in small gaps are stressed by surrounding vegetation and many of them die before they reach reproductive age. Small gaps are sufficient only for gentian seedlings growing in traditionally managed meadows, heathlands and other locations where competition is less and smaller gaps are created regularly.

Acknowledgements. The research was supported by the Grant Agency of the Czech Academy of Sciences (junior grant GA AV ČR 6141901), FRVŠ 0334, and the Mattoni Award. I would like to thank my supervisor Jan Lepš to help me in all difficult steps of this project and my mother, Iva Špačková, Martin Konvička, Priscilla and Jonathan Titus for help with field work and Jan Kučera to help me with determination of mosses. I am grateful to Jitka Klimešová for her comments and to Jonathan Titus for correcting English.

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Appendix 1

List of species in figures of RDA analysis

AgroCani	*Agrostis canina*
AchiPtar	*Achillea ptarmica*
BetoOffi	*Betonica officinalis*
BrizMedi	*Briza media*
CarxDian	*Carex diandra*
CarxHart	*Carex hartmanii*
CarxPale	*Carex pallescens*
CarxPani	*Carex panicea*
CarxPuli	*Carex pulicaris*
CarxUmbr	*Carex umbrosa*
CirsPalu	*Cirsium palustre*
DantDecu	*Danthonia decumbens*
EquiPalu	*Equisetum palustre*
FestRubr	*Festuca rubra*
FranAlnu	*Frangula alnus*
GaliPalu	*Galium palustre*
GaliUlig	*Galium uliginosum*
HolcLana	*Holcus lanatus*
JuncCong	*Juncus conglomeratus*
LathPrat	*Lathyrus pratensis*
LuzuMult	*Luzula multiflora*
LysiVulg	*Lysimachia vulgaris*
MoliCaer	*Molinia caerulea*
MyosNemo	*Myosotis nemorosa*
NardStri	*Nardus stricta*
PediSylv	*Pedicularis sylvatica*
PoteErec	*Potentilla erecta*
PrunVulg	*Prunella vulgaris*
RanuAcri	*Ranunculus acris*
RanuAuri	*Ranunculus auricomus*
RanuNemo	*Ranunculus nemorosus*
SanqOffi	*Sanquisorba officinalis*
ScorHumi	*Scorsonera humilis*
SeliCarv	*Selinum carvifolia*
SuccPrat	*Succisa pratensis*
VeroOffi	*Veronica officinalis*
ViciCrac	*Vicia cracca*

Bryophytes

AulaPalu	*Aulacomnium palustre*
PolyComm	*Polytrichum commune*
RhytSqua	*Rhytidiadelphus squarrosum*
SclePuru	*Scleropodium purum*
SphaFlex	*Sphagnum flexuosum*

Chapter 5

Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L.

Relationships between Population and Habitat Characteristics and Reproduction of the Rare *Gentiana pneumonanthe* L.

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Abstract: *We investigated the relationships among a number of population and site characteristics and the ovule and seed production and seed set in 17 populations of the rare, self-compatible, bumblebee-pollinated perennial* Gentiana pneumonanthe *L. in the Netherlands. Population size and offspring heterozygosity were used as population properties; grass and heath cover, soil concentrations of various ions, soil acidity (pH), and soil moisture were used as habitat characteristics. Our aim was to find out how important population size and genetic variation are for the seed production of a rare plant, when accounting for a number of habitat parameters that might also be important for successful reproduction. Correlation coefficients between population and habitat characteristics showed that sites with low soil moisture and higher concentrations of most nutrients have a higher cover of grasses and a low cover of heath species. At such sites the population size of* G. pneumonanthe *was small and offspring heterozygosity was low. The number of ovules per fruit (a component of reproductive effort) was negatively associated with heterozygosity, phosphorus, and pH and positively associated with calcium, potassium, SO_4, NH_4, and electric conductivity. Total vegetation cover correlated positively with the variation in ovule production among individuals. Seed set and number of viable seeds per fruit, indicators of the success of fertilization and seed maturation, correlated positively with population size, the cover of co-flowering heath species, and the same group of nutrients that (independently) had a positive effect on ovule production. A high NO_2 concentration might be toxic because it had a negative effect on the number of viable seeds. Seed weight seemed to be independent of any population or habitat parameter. Besides lower mean values, we also found more variation in reproductive success among individuals in smaller populations. We conclude that plants in small populations of* G. pneumonanthe *have a similar reproductive potential per flower but have lower seed production and a higher uncertainty of successful reproduction than plants in large populations. Genetic variation was apparently not very important. In addition to population size, habitat characteristics also seemed to be important for seed production and seed set. The most important factors were the facilitating effect of the presence of other insect-pollinated species and the concentrations of specific nutrients in the soil. To restore small populations, we advise small-scale sod cutting around the remaining reproductive individuals. This simultaneously allows germination and seedling establishment of* G. pneumonanthe, *removes soil nutrients, and restores the cover of pollinator-attracting heath species at the cost of grasses.*

Relaciones entre Características Poblacionales y del Habitat y la Reproducción de la Especie Rara *Gentiana pneumonanthe* L.

Resumen: *Investigamos las relaciones entre un número determinado de características poblacionales y de sitio y la producción de óvulos y semillas en 17 poblaciones de la especie de hierba rara, autocompatible, polinizada por abejas y perenne* Gentiana pneumonanthe *L. en los Países Bajos. Utilizamos el tamaño poblacio-*

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Paper submitted February 18, 1997; revised manuscript accepted November 11, 1997.

Conservation Biology, Pages 1042–1053
Volume 12, No. 5, October 1998

nal y la heterocigosidad de la progenie como propiedades poblacionales, la cobertura de gramíneas y ericáceas en la vegetación y la concentración de algunas sustancias nutritivas en el suelo fueron usadas como características bióticas y abióticas del hábitat. Nuestra meta fue la de encontrar que tanta importancia tienen el tamaño poblacional y la variación genética en la producción de semillas de una planta rara, tomando en consideración un número de parámetros del hábitat que pueden ser importantes para una reproducción exitosa. Los coeficientes de correlación entre las características poblacionales y del hábitat mostraron que los sitios con grado de humedad bajo y concentraciones de nutrientes altas tienen una cobertura de gramíneas elevada y de ericáceas reducida. En tales sitios, el tamaño poblacional de G. pneumonanthe *es más pequeño y la heterocigosidad de las progenies es más baja. La producción potencial de semillas (medida en forma de número de óvulos por fruto) tenía una relación negativa con la heterocigosidad, la concentración de P y el pH del suelo y una relación positiva con las concentraciones de Ca, K,* SO_4 *y* NH_4 *y la conductividad eléctrica. Se encontró una correlación positiva entre la variación del número de óvulos y la cobertura total de la vegetación. El éxito de la fecundación y la maduración de semillas estuvo correlacionado positivamente con el tamaño poblacional, la cobertura de las ericáceas (que florecen simultáneamente) en la vegetación y el mismo grupo de nutrientes que (independientemente) surtían efecto positivo en el número de óvulos. La concentración de* NO_2 *en el suelo mostró una correlación negativa con el número de semillas viables. También la variación en el éxito reproductivo fue más alta en las poblaciones pequeñas. No se encontró relación significativa entre el peso de las semillas y alguna de las características de la población o del hábitat. En base a relaciones observadas, concluimos que las poblaciones pequeñas de* Gentiana pneumonanthe *tienen el mismo potencial reproductivo por flor, pero tienen una menor producción de semillas y una mayor incertidumbre reproductiva en comparación con las poblaciones grandes. Los factores importantes para el éxito reproductivo son el tamaño poblacional, el efecto facilitador de la presencia de otras especies de hierbas polinizadas por insectos y la disponibilidad de sustancias nutritivas específicas en el suelo. Recomendamos la eliminación en pequeña escala del césped en los alrededores de los adultos reproductivos remanentes para restaurar pequeñas poblaciones. Este método hace posible al mismo tiempo la germinación y el establecimiento de individuos nuevos, remueve nutrientes del suelo y restablece la covertura de especies atrayentes de polinizadores.*

Introduction

During the last decades, the possible negative consequences of population isolation and reduction for the viability of species have been researched by many conservation biologists throughout the world. It has been demonstrated that small populations experience loss of genetic variation through genetic drift and inbreeding, reduced offspring fitness, increased sensitivity to environmental and demographic stochasticity, and a reduction in seed production and seed set (for reviews see Ellstrand & Elam 1993; Oostermeijer et al. 1996*a*; Young et al. 1996). Many wild plant species depend on interactions with insects, birds, or mammals for the successful pollination of their flowers (Faegri & van der Pijl 1979). Such often delicate interactions can easily be disrupted by the drastic changes in population and habitat characteristics brought about by human activities (Vogel & Westerkamp 1991).

First, reduced reproductive success in small populations may be caused by pollen limitation as a result of reduced visitation by pollinators (Jennersten 1988; Campbell & Halama 1993). Pollinators may lack the ability to locate a small number of flowers at a low density at all, or they may not regard it as a major food source. At many sites, moreover, the total diversity of insect-pollinated plant species has decreased so much that they attract very few pollinators anyway (Rathcke 1983).

Second, increased selfing in small populations may lead to higher rates of seed abortion (i.e., there is inbreeding depression of seed set). Increased selfing may be caused by higher rates of *geitonogamy*, the transfer of pollen between flowers of the same individual (De Jong et al. 1992) or by *autogamy*, spontaneous self pollination within flowers without visitation by pollinators. For self-incompatible species, the absence of suitable mating types in small populations may reduce the production of viable seeds (Reinartz & Les 1994; Luijten et al. 1996).

Third, changes in nutrient or water availability may have occurred in the deteriorated habitats, affecting the number of ovules produced and the patterns of fruit and seed abortion (Casper 1984; Lee & Bazzaz 1986; Campbell & Halama 1993; Martin & Lee 1993; Menges 1995).

In an extensive population viability analysis of the rare *Gentiana pneumonanthe*, we have found that not only population size and genetic variation but also habitat characteristics are very important. Population size was positively correlated with genetic variation, weakly in the maternal plants (Raijmann et al. 1994) but more pronounced in their offspring, probably as a result of increased selfing rates (Oostermeijer et al. 1992, 1994*a*;

Raijmann et al. 1994). Offspring fitness of small populations was also comparatively low (Oostermeijer et al. 1994*a*).

For the pollination of *G. pneumonanthe*, the biotic environment may be very important. Petanidou et al. (1995*a*) found reduced seed set in vegetation patches dominated by purple moor-grass (*Molinia caerulea* (L.) Moench.) compared to patches where other bumblebee-pollinated plant species, such as *Erica tetralix* L. and *Calluna vulgaris* (L.) Hull, were also present. We hypothesized that the presence of other bee-pollinated species in the neighborhood may facilitate the pollination of *G. pneumonanthe* by attracting pollinators.

Molinia caerulea replaces heath species when nutrient availability in the soil increases (by atmospheric deposition) or when the water table is lowered for agricultural purposes (Berendse & Aerts 1984; Heil & Bruggink 1987). On the other hand, individual plants of *G. pneumonanthe* in *Molinia*-dominated patches tend to be larger and have a higher mean number of ovules per ovary. This effect may compensate for any small reductions in seed set (Petanidou et al. 1995*a*; J.G.B.O., S. Borst, and J. Fehse, unpublished data).

The dominance of *Molinia caerulea* at many sites also has major consequences for the demography of *G. pneumonanthe*. The presence of bare soil in the vegetation was positively associated with seedling densities. In vegetation types with a closed structure, populations are "regressive" because they consist only of adult individuals (Oostermeijer et al. 1994*b*, 1996*b*).

Our aim was to investigate the relative importance of population characteristics, such as the number of individuals and the amount of genetic variation, and habitat conditions, such as the cover of grasses and of other insect-pollinated species, the ground water table, and soil chemistry, for the reproductive success of *G. pneumonanthe*. We studied 17 populations of varying size from sites with visually different degrees of environmental degradation. As a result these sites varied considerably in their population and habitat characteristics, enabling an analysis of relationships with components of reproductive success.

Methods

Sampling Procedures and Determination of Reproductive Success

In each of 17 populations of *Gentiana pneumonanthe*, which ranged in size from 5 to over 50,000 flowering individuals (Table 1), we sampled ripe but still closed fruits in September 1994. We took fruits from the top of the inflorescence, if possible, to reduce the probability of sampling late flowers, which tend to have very low fruit set (Petanidou et al. 1995*a*). The sample size depended on the number of fruiting individuals present in the populations at the time of sampling. In the smallest populations we sampled one fruit from each of the flowering individuals. In the large populations we took one fruit from each of 20 different, randomly chosen mother plants. To reduce differences in sample size, we eventually used 16 fruits from each of the large populations and 5–10 fruits from each of the smallest populations.

For 15 populations we used data on allozyme heterozygosity, measured during earlier studies on seed samples raised in the greenhouse (Oostermeijer et al. 1994*a*; Raijmann et al. 1994). Heterozygosity was determined by assaying 40 offspring per population for the following seven enzyme loci: *Aat-1* and *Aat-3* (EC-2.6.1.1), *Mdh* (EC-1.1.1.37), *Nadh.dh* (EC-1.6.99.3), *Ugpp-2* (EC-2.7.7.9), *Pgm-1* (EC-5.4.2.2), and *6Pgdh-2* (EC-1.1.1.44). These loci appeared to be variable after more than 40 enzyme systems were screened in 25 Dutch populations (Raijmann et al. 1994). For the exact electrophoresis procedures, we refer to Raijmann et al. (1994). Although we did not directly analyze the actual seed samples of this project, our earlier data provided an estimate of the amount of genetic variation present in the populations studied.

On each fruit we determined the following parameters: (1) number of viable (filled and well-developed) seeds, (2) number of empty, shriveled seeds (unfertilized or aborted before or after fertilization), (3) number of ovules (sum of the viable and aborted seeds that were recovered in the fruit), (4) ratio between viable seeds and number of ovules (seed set), and (5) weight of a batch of 100 viable seeds randomly selected from each fruit. Seeds were counted under a dissection microscope and weighed on a microbalance.

Vegetation Description and Soil Analysis

On each site a description of the vegetation in the center of the gentian population was made in a plot of 4 × 4 m^2. Each plant species in the plot was scored for relative abundance using a modified Tansley-scale (Tansley 1946): 1, rare (1–2 stems [genets or ramets] occurring in the plot); 2, occasional (3–5 stems); 3, frequent (5–25 stems); 6, abundant (>25 stems); 7, co-dominant (with another species); and 8, dominant. In each plot we also estimated the percent cover of all grasses and of the heath species *Erica tetralix* and *Calluna vulgaris*. Estimation of cover percentages was made with 5% intervals for covers over 10% and with 1% intervals for covers under 10%.

[illegible]

With a small soil corer, we took 35 core samples (diameter 1 cm and depth 2 cm) at random from each location and put them together in a plastic bag. The cores of each location were dried in an oven at 70° C, sieved, and thoroughly mixed. Subsequently, we mixed 20 g of dry soil from each sample with 100 mL of demineralized wa-

Table 1. Population characteristics and habitat characteristics of the 17 sampling sites, with mean values (±SE) of the measured parameters of reproductive investment (number of ovules), reproductive output (number of viable seeds), and reproductive success (seed set and seed weight).

	Population characteristics[a]		*Habitat characteristics*[b]															
Population	*Size* (N_{fl})	H_o *(%)*	*MV*[b]	*Cover of heath (%)*	*Cover of grasses (%)*	*EC*[c] *(μS/cm)*	*pH*	NH_4 *(mg/g)*	NO_3 *(mg/g)*	NO_2 *(mg/g)*	PO_4 *(mg/g)*	SO_4 *(mg/g)*	*Ca (mg/g)*	*K (mg/g)*	*No. ovules/fruit*	*No. viable seeds/fruit*	*Seed set/fruit (prop.)*	*Seed weight/fruit (mg/100)*
Lochem-III	5	0.293	3.5	0.04	0.78	104	4.2	34.0	5.8	0.60	24.0	127	6.4	64	583 ± 61	13 ± 9	0.02 ± 0.01	—[d]
Lange Ven	8	0.229	3.7	0.16	0.35	94	4.6	32.0	10.8	0.04	6.9	71	4.9	51	428 ± 34	267 ± 52	0.60 ± 0.09	3.6 ± 0.4
Lochem-II	12	0.314	3.3	0.06	0.82	118	4.2	31.0	5.3	0.08	7.6	166	8.1	59	316 ± 20	147 ± 51	0.50 ± 0.18	5.0 ± 1.1
Anserdennen	15	0.357	3.7	0.12	0.77	67	4.6	24.0	2.2	0.14	6.6	82	6.7	37	319 ± 23	252 ± 28	0.79 ± 0.06	4.6 ± 0.3
Houtbeek	30	0.200	3.7	0.05	0.82	57	4.4	17.0	3.6	0.10	5.3	76	3.6	31	483 ± 43	269 ± 52	0.53 ± 0.08	4.5 ± 0.3
Leggelderveld	50	—[d]	3.0	0.40	0.52	114	4.2	33.0	7.1	1.30	2.1	121	5.9	59	396 ± 36	289 ± 36	0.75 ± 0.07	4.2 ± 0.3
Drinkput	75	0.317	3.2	0.41	0.50	34	4.7	8.8	1.9	0.06	0.3	25	2.0	23	501 ± 44	343 ± 55	0.68 ± 0.07	3.5 ± 0.2
Kraanlanden	85	0.291	3.6	0.01	0.89	217	4.1	81.0	4.6	0.08	2.8	319	45.0	120	723 ± 34	616 ± 31	0.86 ± 0.03	3.8 ± 0.2
Dwingeloo-II	150	0.319	3.0	0.06	0.92	131	4.4	46.0	3.0	0.07	12.9	113	4.0	67	346 ± 37	249 ± 37	0.74 ± 0.09	4.1 ± 0.2
Zuiderheide	150	0.339	2.9	0.33	0.60	90	5.0	19.5	15.6	0.20	0.7	67	3.4	40	369 ± 28	248 ± 30	0.69 ± 0.07	4.4 ± 0.3
Zeegse Duinen	175	0.329	3.0	0.14	0.55	130	4.4	52.0	2.4	0.09	3.5	112	18.8	68	530 ± 20	382 ± 32	0.73 ± 0.06	4.3 ± 0.3
Dwingeloo-I	250	0.304	2.9	0.42	0.36	52	4.4	9.1	2.1	0.04	2.0	66	4.1	22	445 ± 23	374 ± 25	0.85 ± 0.04	4.1 ± 0.3
Havelte	250	0.300	3.0	0.19	0.53	110	4.6	42.0	2.5	0.14	4.2	107	12.1	51	381 ± 32	327 ± 34	0.85 ± 0.03	4.9 ± 0.2
Assel	500	0.443	3.0	0.14	0.75	96	4.3	25.0	0.9	0.03	2.0	73	4.3	49	432 ± 43	298 ± 43	0.67 ± 0.07	3.7 ± 0.4
Heidebloem	5,000	0.304	3.3	0.26	0.57	103	4.3	14.0	4.4	0.19	4.7	87	6.2	58	452 ± 29	351 ± 30	0.78 ± 0.05	4.7 ± 0.2
Blauwe Bos	>25,000	—[d]	3.5	0.50	0.28	46	4.6	19.0	1.0	1.40	1.3	39	1.7	27	424 ± 20	345 ± 27	0.81 ± 0.05	4.0 ± 0.2
De Deelen	>50,000	0.476	3.4	0.01	0.79	178	4.1	41.0	3.4	0.02	0.7	171	13.4	57	532 ± 37	383 ± 35	0.74 ± 0.06	4.2 ± 0.2

[a] *Size, number of flowering plants;* H_o*, observed offspring heterozygosity.*
[b] *MV, moisture value, and an increasing MV indicates a drier soil.*
[c] *EC, electric conductivity.*
[d] *No data available.*

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ter. We left these mixtures for 2 days, shaking them occasionally. We measured electric conductivity (EC, in µS/cm) and pH(H_2O) on the resulting suspension with a Consort P407 Solution Analyzer. Concentrations of nitrate ($N{\cdot}NO_3$), nitrite ($N{\cdot}NO_2$), ammonium ($N{\cdot}NH_4$), phosphate ($P{\cdot}PO_4$), sulphate (SO_4), calcium (Ca), and potassium (K) were determined colorimetrically with a Technicon-II Autoanalyzer, according to standard procedures (Allen 1974).

Data Analysis

Because of occasional rainfall between days of sampling, large variances would have resulted if we had measured soil moisture directly from the cores. Therefore, we compared the soil moisture levels of the study sites indirectly, using a database with moisture indicator values of the plant species present at each site (Ellenberg-values, modified for The Netherlands by Runhaar et al. [1987] from a large database of Dutch vegetation samples). This approach is frequently used in ecological studies in Europe. We calculated an estimate of the average soil moisture conditions at each site by averaging the indicator values of the species occurring in the plot, weighted by their numerical Tansley score. The weighting by abundance reduces the contribution of incidental species, which may be less characteristic for the moisture conditions at a site. The soil moisture values were assigned the following codes: 1, aquatic; 2, waterlogged soils; 3, wet soils; 4, moist soils; 5, moderately dry soils; and 6, dry soils. According to this scale, when a species has a higher indicator value for soil moisture it shows a preference for drier soils (Runhaar et al. 1987).

Because the various soil parameters were partly intercorrelated, we performed a principal components analysis to reduce the number of parameters to a set of uncorrelated principal components, each representing either a linear combination of a correlated group of parameters or a single variable. To obtain a simpler, more clearly defined structure of component loadings over components, we further rotated the data by the Varimax method (Harman 1976). The sample scores for each of the rotated principal components were used in the statistical analyses.

We studied the relative importance of the population and habitat parameters for the ovule and seed production of *G. pneumonanthe* by means of multiple regression analyses (Sokal & Rohlf 1981). As the independent variables in these analyses, we used the population characteristics of population size and heterozygosity and the habitat characteristics of percent cover of grasses, percent cover of heather, and the rotated scores for the five soil principal components. As dependent variables, we used the number of ovules, number of viable seeds, seed set, and seed weight.

Multiple regression models examine the relationship between a dependent and an independent variable when all other independent variables in the model are (statistically) held constant (Sokal & Rohlf 1981). In a way similar to that described above, we also examined the effects of population size and vegetation composition on the population coefficients of variation (CV) of the number of ovules, the number of viable seeds, and the seed set. This may yield an estimate of the (un)certainty of reproduction in relation to the mentioned parameters. To improve normality, population size was natural log (ln) transformed, and seed set was angularly ($\arcsin\sqrt{\bar{x}}$) transformed prior to all statistical analyses.

Results

Population and Habitat Characteristics

We found considerable variation among the populations in offspring heterozygosity (0.200–0.476) and in the habitat characteristics measured in the field (Table 1). In some sites the cover of heath species was much less than the grass cover, whereas in other sites the situation was reversed (Table 1). Although the values of electric conductivity were all under 200 µS/cm, indicating nutrient-poor soils, variation among sites ranged between 30 and 178 µS/cm. Similar patterns were found for the other soil parameters. Moisture values varied the least (2.9–3.7). All sites could be considered wet to moist, although the highest values were mainly found in the small populations (Table 1).

The reproductive parameters of *G. pneumonanthe*

Table 2. Rotated loadings (Varimax rotation) of a principal components analysis on a correlation matrix of nine parameters determined on soil samples from 17 populations of *G. pneumonanthe*.

	Rotated principal component[a]				
Soil parameter	*1*	*2*	*3*	*4*	*5*
$N{\cdot}NH_4$	0.967	−0.031	0.106	−0.005	−0.074
Ca	0.959	−0.076	−0.109	−0.126	−0.056
K	0.938	0.094	0.125	−0.031	−0.222
SO_4	0.921	0.054	0.011	−0.078	−0.311
EC	0.876	0.140	−0.003	−0.115	−0.354
$N{\cdot}NO_3$	0.067	0.985	−0.016	0.015	0.155
$P{\cdot}PO_4$	0.035	−0.018	0.994	−0.077	−0.062
$N{\cdot}NO_2$	−0.109	0.014	−0.078	0.991	−0.004
pH	−0.452	0.287	−0.101	−0.019	0.838
Variance explained[b]	4.574	1.092	1.044	1.025	1.010
(in % of total)	(50.8)	(12.1)	(11.6)	(11.4)	(11.2)

[a] *Underlined loadings indicate which parameters are significantly correlated with a given principal component.*

[b] *Only principal components with an eigenvalue of 1 or above are presented. The percentage of the total variance explained by each component is given in parentheses. Together, the five principal components explain 97.2% of the total variance.*

Table 3. **Matrix of Pearson's product-moment correlation coefficients between the population and habitat characteristics used to explain variation in the reproductive success of *G. pneumonanthe*.**

	Characteristic^a			
	Population size	*Moisture number*	*Cover of grasses (%)*	*Cover of heath (%)*
Heterozygosity	0.633^{***}	—	—	—
Moisture number	−0.099 ns	—	—	—
Cover grasses (%)	-0.229^{**}	0.294^{***}	—	—
Cover heath (%)	0.093 ns	-0.446^{***}	-0.869^{***}	—
PC 1 (EC, Ca, K, SO_4, NH_4)^b	−0.041 ns	0.193 ns	0.477	-0.584^{***}
PC 2 (NO_3)	-0.239^{**}	−0.128 ns	−0.003 ns	0.057 ns
PC 3 (PO_4)	-0.417^{***}	0.232^{**}	0.351^{***}	-0.295^{***}
PC 4 (NO_2)	0.211 ns	0.026 ns	-0.404^{***}	0.468^{***}
PC 5 (pH)	−0.200 ns	0.047 ns	-0.252^{***}	0.309^{***}

^{a}*, $p \leq 0.05$; **, $p \leq 0.025$; ***, $p \leq 0.01$; *ns, not significant. Significance levels were determined by Bonferroni criteria.*
^{b}*See Table 2 for a further explanation of the soil principal components (PC 1–5).*

were highly variable among populations as well (Table 1). Variation was smallest for the mean number of ovules, which ranged from 316 to 723 per fruit. The viable seed production, however, varied between 13 and 616, resulting in a similarly wide range of seed sets (2–86%).

The soil parameters were subjected to a principal components analysis with Varimax rotation to reduce the number of variables (Table 2). The nine original variables were reduced to five rotated principal components, which together explained most (97.2%) of the total variance. The first component, with an eigenvalue of 4.574, was composed of five of the original variables ($N{\cdot}NH_4$, Ca, K, SO_4, and electric conductivity) and hence explained most of the total variance (50.8%). The other four components mainly represented single variables, which apparently were not strongly correlated with any of the other soil parameters (Table 2).

As known from our earlier studies, population size and offspring heterozygosity were positively correlated (Table 3). Population size correlated negatively with the cover of grasses in the vegetation and with PC 2 and PC 3, which represent the concentrations in the soil of $N{\cdot}NO_3$ and $P{\cdot}PO_4$, respectively (Tables 2 & 3). Increasing grass cover was associated with decreasing cover of the heath species *Erica tetralix* and *Calluna vulgaris*. The moisture value correlated negatively with heath, and positively with grass cover. Heath and grass cover were also complementarily correlated with all soil principal components except for PC 2 ($N{\cdot}NO_3$). From the above relationships, we inferred that higher nutrient lev-

Table 4. **Multiple regression analysis of population (size and heterozygosity) and habitat (vegetation structure and soil factors) variables on three parameters of reproductive success of *G. pneumonanthe*.***

					ANOVA			
Variable	β	t	p	*Source*	*df*	*MS*	F *ratio*	p
Number of ovules per fruit (n = 185, r = 0.557, R^2 = 0.311)								
Heterozygosity	−0.215	−3.179	0.002	regression	4	370083.9	20.293	≤0.001
PC 1	0.459	7.394	≤0.001	residual	180	18237.3		
PC 3	−0.277	−4.198	≤0.001					
PC 5	−0.230	−3.610	≤0.001					
Number of viable seeds per fruit (n = 216, r = 0.725, R^2 = 0.526)								
No. ovules/fruit	0.575	10.720	≤0.001	regression	5	638599.8	46.518	≤0.001
Population size	0.134	2.720	0.007	residual	210	13728.0		
Cover of heath (%)	0.281	3.912	≤0.001					
PC 1	0.344	5.022	≤0.001					
PC 4	−0.144	−2.394	0.018					
Seed set per fruit (n = 216, r = 0.308, R^2 = 0.095)								
Population size	0.143	2.180	0.030	regression	3	0.896	7.396	≤0.001
Cover heath (%)	0.277	3.425	0.001	residual	212	0.121		
PC 1	0.303	3.767	≤0.001					

**Parameters are number of ovules per fruit, number of viable seeds per fruit, and seed set (ratio of viable seeds to ovules). Only the significant regression models with their constituent parameters are shown. Abbreviations: β, standardized partial regression coefficient; r, multiple correlation coefficient; R^2, squared multiple r (≈ proportion variance explained by the model); n, number of samples used in analysis; df, degrees of freedom; MS, mean square.*

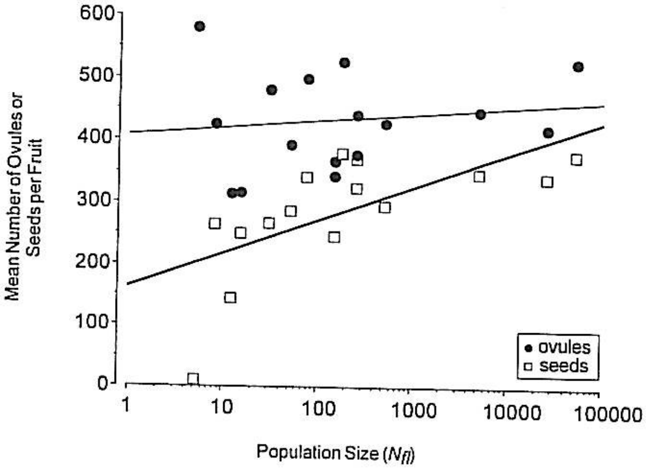

Figure 1. Univariable relationships between population size and the mean number of ovules and seeds per fruit. A regression line was fitted through each set of data. Ovule no. = 407 + 5 × log(size), $R^2 = 0.031$, $p = 0.511$, *seed no. = 162 + 24 × log(size),* $R^2 = 0.444$, $p = 0.005$. *Population size is given as number of flowering plants (*N_{fl}*).*

els and a drier soil were associated with a lower cover of heath and an increasing dominance of grasses in the vegetation. In relatively dry, nutrient-rich situations in which grasses were dominant, population size of *G. pneumonanthe* was often small, and offspring heterozygosity tended to be reduced.

Population and Habitat Characteristics and Reproductive Success

We found significant multiple regression models for the relationships between the population and habitat characteristics and the number of ovules, the number of viable seeds, and seed set (Table 4). No significant model was found for seed weight. Seed production was correlated with population size, but ovule number per fruit was not (Fig. 1). Especially populations with less than approximately 100 flowering plants had reduced seed production compared to larger populations.

The number of ovules (Table 4) significantly correlated with three of the five soil principal components (PC 1 [+], 3 [−], 5 [−]; Table 2). This indicates that the number of ovules increases with electric conductivity and the concentrations of ammonium, calcium, potassium, and sulphate in the soil (PC 1). The number declines, however, when the concentration of phosphate (PC 3) and the soil pH (PC 5) increase. Heterozygosity was negatively associated with ovule production per fruit. This suggests that, when all other variables are kept constant, individuals in genetically more variable populations tend to produce fewer ovules per fruit.

The number of viable seeds is a combination of the reproductive potential and successful fertilization and maturation of seeds. Because the number of viable seeds partly depends on the initial number of ovules per flower, we included the latter parameter as an independent variable in the multiple regression (Table 4). As expected, the initial number of ovules explained much of the variance in the number of viable seeds. Seed production, however, was also positively correlated with population size (Fig. 1) and with the cover of heath species in the vegetation. Judging from the standard partial regression coefficients (β), heath cover is even more strongly associated with seed production than population size (Table 4). Also, the soil parameters PC 1 (EC, Ca, K, SO_4, and NH_4) and PC 4 (NO_2) are correlated with seed production. PC 1 has a positive and PC 4 a negative effect, however.

Seed set—the proportion of ovules that developed into viable seeds—is a measure of successful fertilization and maturation of seeds and is independent of the initial number of ovules. As with the number of viable seeds, this parameter also appeared to be positively related to population size and even more strongly to the percent cover of the co-flowering heath species, *E. tetralix* and *C. vulgaris* (Table 4). Also, the concentration of various nutrients in the soil (PC 1) contributed significantly to the model. The proportion of the total variation in seed set that was explained by the regression was clearly lower than for the previous two models (9.5%, compared to 31.1% for the number of ovules and 52.6% for the number of viable seeds).

The variation between individuals within populations in the number of ovules was not related to population size but was positively related to the *combination* of grass and heath cover (single regressions with these parameters separately were not significant). This implies that when either grass or heath cover is held constant, an increase in the cover of the other parameter results in a higher variation of ovule number in a population. This suggests that the total vegetation cover is more important than which species make up the vegetation.

Smaller population size associated significantly with higher CV for both the number of viable seeds and seed set (Table 5). For these parameters vegetation cover apparently did not play an important role.

Discussion

Population Size and Habitat Characteristics

The dominance of grasses or heath species in the vegetation seemed to be largely associated with the concentration of nutrients in the soil and the soil moisture level. At high nutrient levels and low soil moisture, grasses were dominant in the vegetation and the cover of heath species was low. These observations agree with the experi-

Table 5. Multiple regression analysis of population size and grass and heather cover on the population coefficients of variation (CV) of three parameters of reproductive success of *G. pneumonanthe*.*

Variable	β	t	p	*Source*	*ANOVA*			
					df	*MS*	F *ratio*	p
CV ovule number per fruit (n = 17, r = 0.557, R^2 = 0.333)								
Cover grasses	1.029	2.581	0.022	regression	2	0.016	3.498	0.059
Cover heather	0.987	2.476	0.027	residual	14	0.005		
CV number of viable seeds per fruit (n = 17, r = −0.579, R^2 = 0.336)								
Population size	−0.579	2.753	0.015	regression	1	0.251	7.578	0.015
				residual	15	0.033		
CV seed set per fruit (n = 17, r = −0.494, R^2 = 0.244)								
Population size	−0.494	2.202	0.044	regression	1	0.169	4.851	0.044
				residual	15	0.035		

**Parameters are number of ovules per fruit, number of viable seeds per fruit, and seed set (ratio of viable seeds to ovules). Only the significant regression models with their constituent parameters are shown. Abbreviations: β, standardized partial regression coefficient;* r, *(multiple) correlation coefficient;* R^2, *squared (multiple)* r *(≈ proportion variance explained by the model);* n, *number of samples used in analysis; df, degrees of freedom; MS, mean square.*

ments of Berendse and Aerts (1984) and Heil and Bruggink (1987), who found that the grass *Molinia caerulea* replaces *Erica tetralix* and *Calluna vulgaris* when concentrations of nutrients increase and the water table is lowered (often in relation to agricultural land-use nearby). A small population size of *G. pneumonanthe* was significantly associated with dominance of grasses in the vegetation. This is consistent with our demographic studies, which showed that population density was lower in very closed vegetation and that the population stage structure was mostly regressive—seedling recruitment did not occur (Oostermeijer et al. 1994*b*, 1996*a*).

Besides demographic effects of vegetation structure on population size, some physiological effects of soil chemistry on plant performance might exist. We found negative correlations between population size and concentrations of PO_4 and NO_3 (Table 3) that did not disappear when a multiple regression was performed with grass cover included (statistics not shown). This suggests that even when the cover of grasses remains constant, an increasing concentration of NO_3 and/or PO_4 may lower the survival rates of *G. pneumonanthe*. In another heathland species, *Arnica montana* L., increased concentrations of nitrogen in the soil lowered shoot biomass, leaf area, flower and seed production, and seed weight in experiments that included competition of grasses (Fennema 1990). Without competition, however, a moderate increase in nitrogen stimulated the performance of *A. montana*, although very high nitrogen concentrations seemed to have toxic effects (Fennema 1990).

Population and Habitat Characteristics and Reproduction

REPRODUCTIVE POTENTIAL

The average number of ovules that an individual produces per ovary is one component of investment in reproduction. No relationship was found between this measure and population size, indicating that the remaining individuals in small populations could potentially produce as many seeds per flower as plants in large populations. The variation in ovule number per population was positively related to total vegetation cover, however, which suggests that increased competition may affect investment in reproduction more strongly in some plants than in others. Another factor affecting an individual's potential fecundity is the total number of flowers, which was not measured in this study. Frequently, plants in small, regressive populations are slightly larger and produce more flowers than plants in large, stable, or growing populations. This difference arises because turnover of individuals is higher in the latter populations, so the average plant does not reach the size that corresponds with an older population (Oostermeijer et al. 1996*b*). A larger number of flowers can to some degree compensate for the reduced number of seeds per flower in terms of total seed output per plant. Nevertheless compensation for a 50% reduction in seed set calls for twice as many flowers per plant, something that is rarely found. Moreover, flower number only compensates for seed number, not for the reduced seed quality of increased selfing.

Ovule production was negatively related to offspring heterozygosity, positively related to electric conductivity and the concentrations of NH_4, Ca, K, and SO_4 in the soil (PC 1), and negatively related to phosphate concentration (PC 3) and pH (PC 5). The first result was unexpected. In an earlier study we observed an increase in adult size, number of flowers per plant, and flowering percentage with both population and individual heterozygosity (Oostermeijer et al. 1994*a*, 1995*a*), so we expected a similar trend for the number of ovules per flower. One explanation for this unexpected relationship is that in the present study there is no direct connection between the plants on which ovules were counted and those on which heterozygosity was determined. We decided to use the data on heterozygosity as a *population* measure of genetic variation. Therefore, it is difficult to draw conclusions about any direct relationships between heterozygosity and ovule production, which act on the *individual* level. The observed negative correlation may have been caused by various kinds of interactions on the population level, although we can rule out the factors measured that did not significantly contribute to the regression model. One of the variables we did not measure was plant size. Possibly, larger plants produce more ovules per flower. As mentioned, plants in the generally regressive small populations tend to be larger than plants in stable or growing populations (Oostermeijer et al. 1994*b*). Mean size of reproductive individuals tended to increase from 13.2 to 17.5 cm when populations became regressive in the course of vegetation succession (Oostermeijer 1996*b*). At the same time, both population size and outcrossing rate decreased (Raijmann et al. 1994). So, in small, regressive populations, we find large motherplants with more inbred (less heterozygous) offspring. If larger plants have larger flowers with more ovules, this would explain the unexpected negative correlation between offspring heterozygosity and ovule number. The positive correlation between flower size and ovule number was highly significant in an earlier study ($R^2 = 0.123$, $p \leq 0.005$, $n = 64$; J.G.B.O., S. Borst, and J. Fehse, unpublished data).

Easier to explain is the positive correlation between the number of ovules and the concentration of nutrients in the soil. Plants that grow on richer soils may have more resources available to invest in reproduction and thus produce more ovules (Martin & Lee 1993). On the other hand, we have observed that individuals of *G. pneumonanthe* growing in vegetation patches on richer soils dominated by *Molinia caerulea* produce as many ovules as plants in nutrient-poor *Erica*-dominated patches, but they are probably not reallocating the nutrients invested in the ovules by means of resorption of the unfertilized or aborted ones during seed maturation. The absence of resorption leads to a higher recovery of "empty" ovules during counting and thus, seemingly, in a higher number of ovules produced (Petanidou et al. 1995*a*). The same phenomenon may have occurred during our study, leading to a higher recovery of ovules in populations from sites richer in nutrients.

FERTILIZATION AND MATURATION OF SEEDS

Our data support the initial hypothesis that both a relatively large population size and the presence of co-flowering (bumble)bee-pollinated species independently have a positive effect on the reproductive success of *G. pneumonanthe*. The relative contribution of the cover of heath species to the regression model was even larger than that of population size.

Reduced seed set in relation to small population size or low population density has been demonstrated in a number of other rare plant species (Karron 1987; Jennersten 1988; Kwak 1988; Kwak et al. 1991; Lamont et al. 1993; Van Treuren et al. 1994; Boerrigter 1995). As our present data suggest, however, the negative effects of small populations can be less severe if the surrounding vegetation contains a sufficient number of other insect-pollinated species to attract pollinators to the site. This facilitation effect (Rathcke 1983), which we observed in a comparison between different vegetation patches within a large population (Petanidou et al. 1995*a*), thus also seems to occur on a wider scale. Of course, we have no direct proof that more pollinators were present on sites with more heath species. Nevertheless, seed set can be considered a good indicator of the frequency of pollinator visitation in the fully self-compatible *G. pneumonanthe* (Petanidou et al. 1995*a*). Other explanations for reduced seed set could be increased competition by grasses for nutrients and the availability of water or nutrients. These variables, however, were all included in the regression models. Grass cover and moisture values were not significantly related to seed production and seed set, and higher nutrient concentrations had a positive effect. Hence, these factors cannot explain why higher seed set was observed in sites with higher heath cover, especially because these tended to be poorer in nutrients.

The effects of co-flowering species are not always positive, especially when they are much more abundant. Competition for pollinators may then occur as well (Mosquin 1971; Rathcke 1983; Kwak & Jennersten 1991). In the perennial herb *Gentiana cruciata* L. we attributed low seed set in a small population to the fact that bumblebees visited the common co-flowering *Rubus caesius* more frequently than *Gentiana*, so pollen loads were composed of more *Rubus* than *Gentiana* pollen (Petanidou et al. 1995*b*). Judging from a positive relationship between heath cover and seed production and seed set observed in the current study on *G. pneumonanthe*, this kind of competition for pollination—for instance with *Erica tetralix* or *Calluna vulgaris*—did not occur (see also Petanidou et al. 1995*a*).

Population size and the percent cover of co-flowering heath species were not the only parameters that showed a significant positive association with seed production. The concentration of a specific intercorrelated group of nutrients (NH_4, Ca, K, SO_4, and electric conductivity) also contributed significantly to the regression. This suggests that both the investment in reproduction—the initial number of ovules—and the successful fertilization and maturation of seeds may be affected by the resources available in a habitat or microsite. Several experimental studies have provided evidence for this hypothe-

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sis (Janzen 1976; Casper 1984; Lee & Bazzaz 1986; Martin & Lee 1993). The observed negative correlation between seed number and nitrite concentration suggests a toxic effect, which may be similar to the impact of high nitrogen concentrations on *Arnica montana* (Fennema 1990).

Besides being affected by resource availability or toxic compounds, abortion of fertilized ovules can also result from lethal gene combinations in some embryos (Wiens et al. 1987; Lee 1988) or from competition for maternal resources between genetically inferior and superior seeds (Wiens et al. 1987; Marshall & Ellstrand 1988). This genetic explanation of reduced seed set apparently does not apply to *G. pneumonanthe* because the level of offspring heterozygosity—a measure of the amount of inbreeding in the population—was not significantly associated with seed set or the number of viable seeds produced. This is consistent with the observation that seed set in this species is not reduced by the full inbreeding caused by manual self-pollination (Petanidou et al. 1995*a*). On the other hand, we found a significant positive relationship between seed set and the distance of the pollen donor (Oostermeijer et al. 1995*b*). This indicates that a genetic component affecting ovule abortion cannot fully be excluded.

Seed weight was not related to any of the population or habitat parameters in this study. This is in contrast with the positive correlation with population size we found earlier (Oostermeijer et al. 1994*a*). Within one large population we also found that seed weight tends to be lower when seed set is high. This may be explained by allocation of similar amounts of energy to more seeds (Oostermeijer et al. 1995*b*). This relationship was also not observed in the present study, either calculated over all samples ($p = 0.710$) or in any of the populations separately ($p > 0.25$ in all cases).

Smaller population size was also associated with more variation in seed set and, consequently, with variation in seed production. Apparently, individual plants in small populations are less certain of producing a large amount of viable seeds than plants in large populations. This increase in stochasticity may negatively affect the probability of extinction (Menges 1993).

Conclusions

Our data show that although the number of ovules produced per flower is the same, the actual seed production of small populations of *G. pneumonanthe* is reduced, as is the reliability of successful seed production. Although we present only correlative evidence, which should not be interpreted as indicating cause-effect relationships, we can identify various likely causes for the reduced seed production of plants in small populations. First of all, the population size itself may be too small to attract sufficient numbers of bumblebees to the flowers. Second, many small populations are found in habitats where the vegetation is dominated by grasses that have replaced a number of other plant species adapted to insect pollination. This dominance of grasses is clearly associated with increased levels of nutrients and a low water table. It may be expected that vegetation types with few entomophilous species are not attractive to pollinating insects, so many small populations of *G. pneumonanthe* cannot benefit from pollination facilitation.

Besides having negative effects on the composition of the vegetation, the increased levels of some nutrients may also have positive effects on reproductive success: ovule and seed production seemed enhanced in nutrient-rich environments. Negative relationships were also observed, for example, with nitrite concentration. Experimental studies are necessary to unravel the physiological causes of these specific effects.

Genetic variation and outcrossing rates are apparently less important for reproduction than the environmental deterioration described above. The reduced genetic variation and increased selfing rates observed in small populations of *G. pneumonanthe* (Raijmann et al. 1994; Oostermeijer et al. 1994*a*, 1995*a*) do not seem to have any significant effect on reproductive success in terms of the number of offspring produced. Hence, it is mainly the quality and not the quantity of the produced offspring that is negatively affected by inbreeding (Oostermeijer et al. 1994*a*; Oostermeijer 1996*a*,1996*b*).

For successful regeneration in small populations of *G. pneumonanthe*, optimal reproductive success is important. Fortunately, the management strategy of small-scale sod cutting (in the past a commonly used agricultural practice in heathlands) around the remaining adult plants not only creates suitable microsites for germination and seedling recruitment of *Gentiana* (Oostermeijer et al. 1994*b*) but removes excessive amounts of nutrients from the soil and restores dominance of the heath species *Erica tetralix* and *Calluna vulgaris* in the vegetation. Restoration of the latter especially may facilitate pollinator visitation and increase seed set. Of course this effect is mainly quantitative; the low number of individuals and the low density in the remnant population may still cause high selfing rates (through biparental inbreeding or geitonogamy), so that inbreeding depression is not alleviated by this management strategy.

The apparent facilitating effect of the presence of other insect-pollinated plant species in the surroundings of *G. pneumonanthe* raises this study from the population to the community level. Most studies of small populations have focused mainly on population size to explain reduced seed production. We have shown that other factors may also play an important role. Furthermore, our results demonstrate that a detailed understanding of the population ecology of single species can lead to conservation and management measures that affect its whole habitat positively. This effect will of course be greater if the species under study is a good indicator of the overall quality of its habitat.

Acknowledgments

We especially thank the students of the 1994 course in evolutionary biology for their help with counting the thousands of tiny aborted and viable seeds for this study. We also thank R. Bregman for analyzing the soil samples and G. Islebe for correcting the Spanish abstract. E. Menges and two anonymous reviewers provided valuable comments on an earlier version of this paper.

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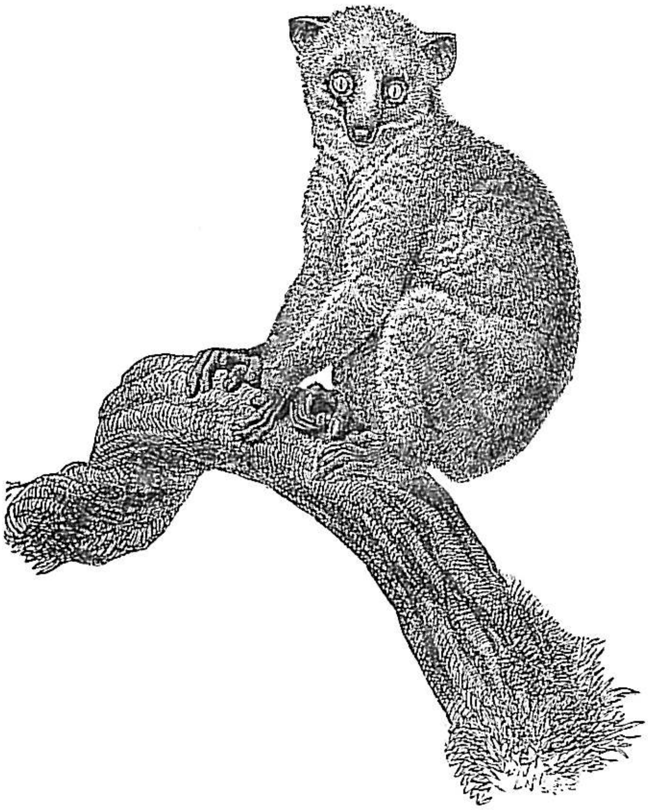

Chapter 6

Factors causing decline and extinction of the Blue Alcon butterfly in the SW of the Czech Republic, Central Europe

Factors causing decline and extinction of the Blue Alcon butterfly in the SW of the Czech Republic, Central Europe

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Will be submitted to Conservation Biology

Abstract: *We investigated more than fifty sites in the SW part of the Czech Republic where Gentiana pneumonanthe, the host-plant of the rare butterfly, Maculinea alcon, grew and found eighteen populations of M. alcon where only one locality was known before 1995. The G. pneumonanthe sites were characterised by a number of ecological characteristics in an attempt to determine key factors in the occurrence of Maculinea alcon. M. alcon populations survive at several isolated sites, with variable plant species composition. We found no significant differences in plant species composition between sites of G. pneumonanthe with and without M. alcon populations. The number of flowering individuals of G. pneumonanthe was the best predictor of the occurrence of M. alcon (p<0.001). We recorded three potential host Myrmica species (Myrmica rubra, M. ruginodis, and M. scabrinodis) at the sites and found the larvae of M. alcon in nests of the most common, M. scabrinodis. The average annual precipitation (p < 0.001) and numbers of rainy days in summer (p < 0.005) are very good predictors of the occurrence of M. alcon. In 1996 we found several new populations of M. alcon and G. pneumonanthe in the southern part of the study area. Since 1997, several of these populations became extinct in this part of the study area. Although we investigated various biotic and abiotic characteristics of these sites, we did not find any significant differences between localities where the butterfly survived and became extinct.*

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Long-term climatic data showed that the annual precipitation and number of days with snow cover was higher and annual average temperature lower in this region in compared with other parts of the study area. Detailed climatic data for 1994 – 1999 illustrate atypical fluctuations in 1996 and 1997. It is likely that climatic conditions caused the extinction of M. alcon populations at the edge of the species climatic range.

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Introduction

The dramatic changes in land use that occurred during the twentieth century caused big changes in the European environment, which led to the widespread decline of many wildlife groups, including butterflies. The decline and local extinction of many butterfly populations, including *Maculinea*, species related to changes in the availability and quality of their habitat (Hanski 1999). All five European *Maculinea* butterflies species are endangered (Wynhoff 1998). The new IUCN list gives four of them 'vulnerable' and *M. arion* 'endangered' status. *Maculinea rebeli* is a species of a global conservation concern because it is restricted to Europe (van Swaay & Warren 1999).

The *Maculinea* species have evolved a phytopredacious life-habit (Fiedler 1994). Similarly as more than 50% of species of the family *Lycaenidea*, the larvae of *Maculinea* butterflies also have a mutualistic association with ants. Their polytrophic interactions have been intensively studied not only from an evolutionary point of view, but as here with the objective of better understanding them so that interactions? they can be for conserved or reintroducted into regions where they have become extinct.

There have been done many studies on the ecology, evolution, genetics, behaviour etc. of *Maculinea* over the last decade (review in Thomas et al 1998a). Most of these studies were done on the edge of the species' ranges, in West European countries. Population modelling of the spatial interactions between *M. rebeli*, their food-plant and host *Myrmica* ants within a site, showed that the spatial pattern of 'suitable' habitat of variable quality ~~within a site~~ can influence the local butterfly population size and perhaps also its persistence (Clarke et al. 1998).

The aim of our study was to define the factors that govern the occurrence of *Maculinea alcon* on a countryside scale (several hundred square kilometres). We also collected information on the ecology of *M. alcon* in Central European habitats, which we supposed is similar to that at the centre of its distribution. Since the species disappeared from several of the localities during the study we also analysed the factors causing the extinction of *M. alcon*.

Material and methods

Study species

The Blue alcon, *Maculinea alcon* (Denis & Schiffermüller 1775), together with *Maculinea rebeli* (Hirschke 1904), were formerly regarded as subspecies of *M. alcon* s.l. and many authors did not distinguish them until the 1980s, although their habitats are clearly different. *Maculinea alcon* and its unique host-plant *Gentiana pneumonanthe* occur in damp, wet oligotrophic grasslands, wet heathlands and bogs in the western part of its distribution where *Erica tetralix* and *Calluna vulgaris* are dominant (Wynhoff et al. 1996) while *M. rebeli* prefers very dry to moist, mostly calcareous grasslands where its host-plant, *Gentiana cruciata*, grows (van Swaay & Warren 1999). Larvae of both species mimic the behaviour of ants larvae and are preferentially fed by the nurse ants. Their cuckoo-feeding strategy distinguishes them from predacious *Maculinea* (*M. arion, M. telius, M. nausithous*), which feed on the offspring of *Myrmica* ants.

Imagoes of *M. alcon* fly for several weeks in July. Their flight period can shift to the end of June or beginning of August as a consequence of climatic or management change. They can fly distances up to 500 m across unsuitable habitats. Females oviposit on the buds and flowers of their host-plant *Gentiana pneumonanthe*. They prefer gentians close to ant nests at the beginning of oviposition but later in the season will oviposit on gentians some distance from ants' nests (van Dyck et al. 2000). The young caterpillars feed inside the marsh gentian capsules. After reaching the fourth and final instar they drop to the ground and wait discovery by *Myrmica* workers. They can move only several centimetres from a host-plant and usually die if they are not close to a *Myrmica* ant nest (Elmes et al. 1991). The probability of their being adopted by ants decreases in time. Workers of all *Myrmica* species find and adopt *Maculinea* caterpillars with equal probability, but the caterpillars only survive in nest of particular *Myrmica* species. Successful survival of *Maculinea alcon* caterpillars was observed in nests of *Myrmica rubra, M. ruginodis,* and *M. scabrinodis* (Elmes et al. 1994). Caterpillars spend nearly eleven months in *Myrmica* nests. Imagoes leave the nests after pupation in July the following year. Originally all *Maculinea* species were considered to be annual. But Thomas et al. (1998a) and Schönrogge et al. (2000) recently demonstrated that *Maculinea rebeli* and *M. alcon* have both annual and biennial life cycles. Some individuals, are faster developers, increase in weight rapidly and pupate within thirty weeks. Others grow slowly and spend more than one year in an ant's nest.

Study area

In Central Europe, meadows at lower altitudes are man-made and maintained. Traditional management practices, mainly hay-making and grazing (Bakker 1989), have maintained these meadows for centuries, but management practices have changed dramatically recently. One of the most rapidly vanishing grassland communities in Central Europe are wet oligotrophic meadows. Their species richness is very high; at the scale of metres they belong to the world's most diverse plant communities (Lepš 1999). Large areas of wet oligotrophic meadows were drained and/or fertilised and became species poor meadows. Many species disappeared and several originally common species now survive only as small isolated populations. Changes in agriculture management (land drainage as well as abandonment) are the main factors that threaten the extinction of *Maculinea alcon* in grassland habitats (van Swaay & Warren 1999).

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This study was carried out in the south-west part of the Czech Republic. The area of interest is about 20 000 km^2. The landscape consists of a mosaic of forests, fields, settlements and semi-natural habitats. Military training areas (MTAs; both recent and former) and the former frontier zone; i.e. a corridor several kilometres wide along the west border ("the iron curtain"), which was closed to people before 1990, are important for the protection of habitats and species. The originally densely inhabited areas were depopulated after War II and were not subject to intensive agriculture management during the second half of the 20th century. Most of the wet species rich meadows were

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not drained and natural succession occurred in many square kilometres of the MTAs. The military activities kept the vegetation open and occasional disturbances (by, e.g. tanks and shell impacts) helped to reverse the vegetational succession. Patches of various habitats create favourable conditions for viable populations of many species, which disappeared from the surrounding countryside. Although the MTAs have not the status of the Nature protection areas, they represent some of the most important conservation areas in Central Europe. The species rich habitats in the Boletice MTA (219.49 km^2) are the most valuable part of our study area. Many rare and endangered butterflies and plant species occur in this area (Pavlíčko 1997).

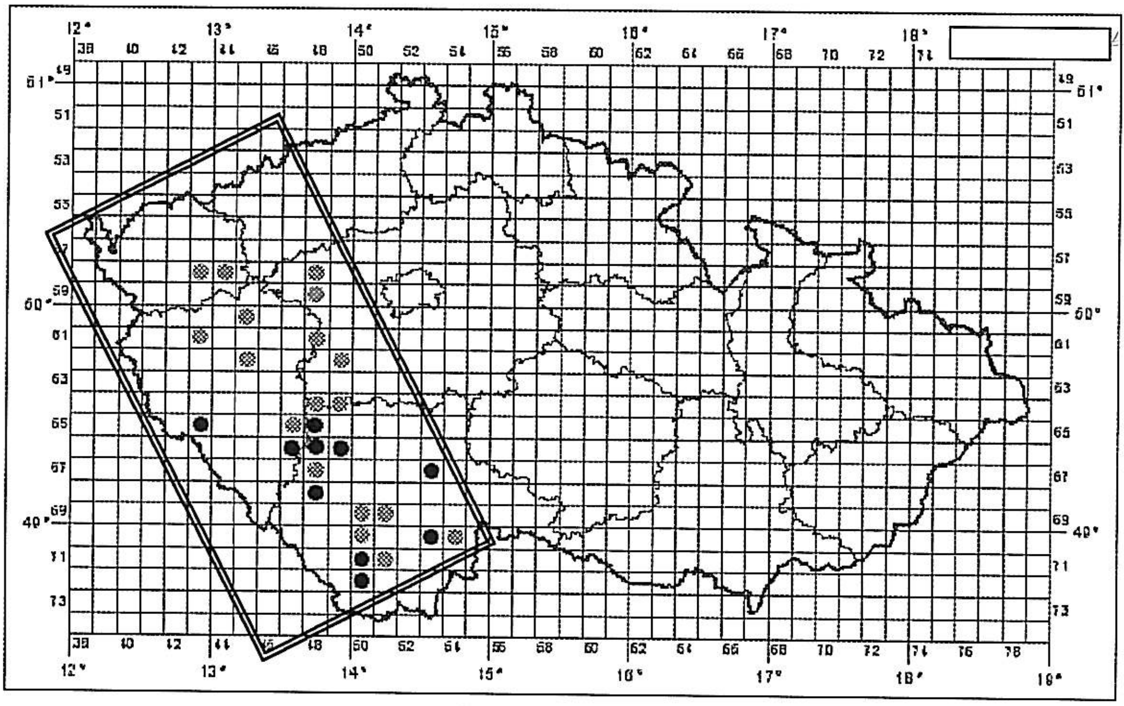

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Figure 1. Standard net map of the Czech Republic with grid 10.5 x 11.2 km. The borders of the counties are displayed and the frame shows the area of interest. The quadrates with sites of Gentiana pneumonanthe *where* Maculinea alcon *was present are displayed as black points and sites without* M. alcon *are displayed as gray points.*

Defining the habitats of *Maculinea alcon*

From 1996 – 2000 we investigated the occurrence of *Maculinea alcon* at recently colonised by sites *Gentiana pneumonanthe* in the South-west part of the Czech Republic.

From the information in the literature, herbaria, and from local managers, conservation authorities, amateur botanists and lepidopterists we prepared a list of known and potential sites for of *G. pneumonanthe*. We visited these sites twice a year – in July and again in August or September. We treated both observations of imagoes and eggs on host-plants as occurrence of *M. alcon* at the site. We recorded all recent sites on a standard net map with grid 10.5 x 11.2 km (Fig. 1) and annually visited them to record biotic and abiotic conditions, which we thought might influence the occurrence of *M. alcon* (Appendix 1).

For all localities we recorded the altitude, distance to the nearest *Maculinea alcon* sites and types of management. We distinguished five management types: grazing, hand mowing, machine mowing, irregular mechanical disturbances (e.g. heavy vehicles tracks, tanks and shell impacts) and unmanaged sites. Climatic conditions at sites we characterised using the following parameters: the average annual temperature, annual precipitation, number of days with temperature < -10°C, number of days with snow cover, number of rainy days in summer (June – August). For this we used the long-term data-set of the Czech Hydrometeorological Institute.

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At all sites we described the vegetation structure in 3x3 m relevés (Zurich-Montpellier system) and total % cover and % bryophyte cover. We characterised the populations of the host-plant, *Gentiana pneumonanthe*, by its population size, height, number of stems and number of flowers of fifteen randomly selected flowering gentians, and population type. The effective population size was the total number of all flowering gentians regardless of their spatial pattern, height and number of flowers. We recorded the height, number of stems and number of flowers of 15 randomly selected individuals. We used the classification of *Gentiana* populations sensu Oostermeijer et al. (1995), who distinguished three population types. A high proportion of seedling and juvenile individuals in populations is typical for the ′invasive′ population type, individuals of all ontogenetic stages are present in populations of the ′stable (or normal)′ population type, while only adults individuals occur in populations of the ′senile′ type. At several sites we recorded the occurrence of *Myrmica* ant nests, their spatial distribution and time dynamics, but we used only the preliminary results of this in this paper.

Factors causing *Maculinea alcon* extinction

In 1996 we found several previously unrecorded populations of *Gentiana pneumonanthe* in the MTA Boletice. *Maculinea alcon* populations were present at some of them. In 1997 we recorded no imagoes or eggs at these sites although we checked them every 10 days and searched most of the gentians for eggs. This was continued in 1998 and 1999.

In 1999 and 2000 we again studied all the biotic and abiotic conditions thought to affect *Maculinea alcon* extinction. Additionally we studied the species diversity, local density and distribution of *Myrmica* ant species in 75 m^2 plots at all sites. We also contacted the managers of the MTAs to get information any change in management of the sites. Finally we used climatic data from the two nearest meteorological stations (12 km north-east and 14 km south of the study area) to determine departures from standard climatic conditions in the region. We used daily records of temperature, precipitation and snow cover in this region in 1994 – 1999.

Data analysis

We used the Canonical Corresponded Analysis (CCA) in the CANOCO package (ter Braak & Šmilauer 1998) to test the differences in species composition, differences among sites and correlation between species composition and occurrence of *Maculinea alcon*. We used the occurrence of *M. alcon* as an explanatory variable and performed the „reverse" analysis (*i.e.*, species composition might influence the occurrence, not vice verse). In all cases, we tested the significance of the results by the Monte Carlo permutation test. We used the programs CanoDraw and CanoPost (Šmilauer 1992, ter Braak & Šmilauer 1998) for graphical presentation of the ordination results.

We used the logistic regression models in the STATISTICA package (Anonymus 1999) to test the effect of the host-plant population size on *M. alcon* occurrence. We tested the effect on *M. alcon* occurrence separately for each year in the period 1996 – 2000 and also for all *M. alcon* sites found during the study period. We also used the logistic regression models to test the effect of the average annual temperature, annual precipitation, number of days with a temperature < -10°C, number of days with snow cover, and number of rainy days in summer on *M. alcon* occurrence.

Results

Defining the habitats of *Maculinea alcon* in Central Europe

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In total we investigated fifty-two sites with *Gentiana pneumonanthe* from 1996-2000 as potential sites for *Maculinea alcon*. We included all the recently recorded sites of the unique host plant in the SW part of the Czech Republic (Fig. 1). We found and studied eighteen populations of *Maculinea alcon* (Fig. 2).

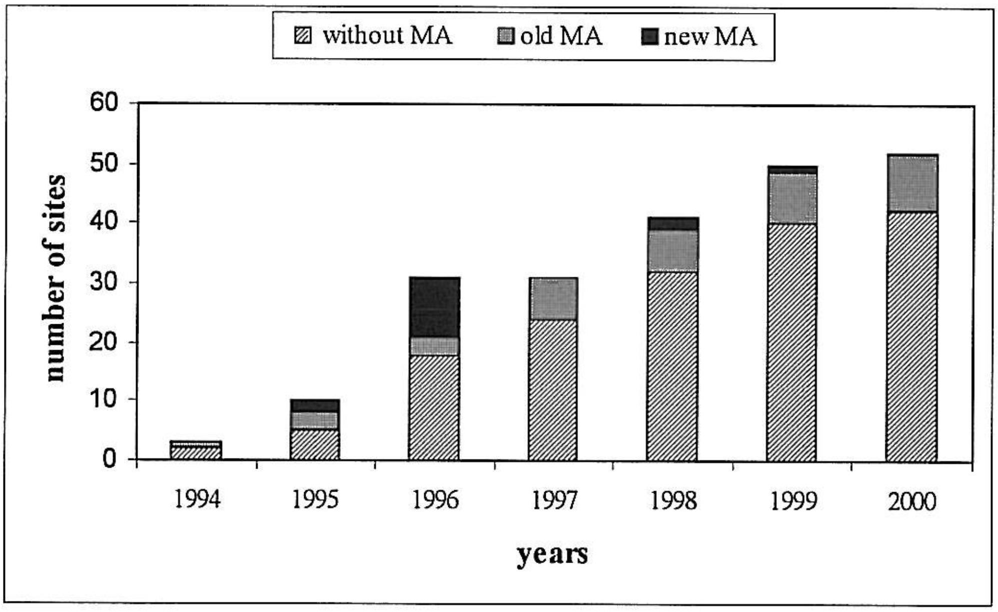

Figure 2. Maculinea alcon *and* Gentiana pneumonanthe *sites studied in the SW part of the Czech Republic in years 1994 - 2000. Detail screening of all known* G. pneumonanthe *sites started in 1996. Data from years 1994, 1995 are uncompleted. In 1994 only one recent site of* M. alcon *was known in the region.*
'without MA' - the sites of G. pneumonanthe *without* M. alcon; *'old MA' - the sites of* G. pneumonanthe, *where occurrence of* M. alcon *was known from the previous year; 'new MA' - the sites of* G. pneumonanthe, *where occurrence of* M. alcon *was found newly.*

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M. alcon populations survived in protected areas, abandoned pastures or rock meadows, and MTAs. Many populations were isolated, with the distance to the nearest population ~~are~~ more than fifteen kilometres. Six populations were concentrated in sparsely inhabited borders between two counties (Fig. 1). Distances between them were only several kilometres. Distances between the five *M. alcon* populations on the MTA Boletice were only several hundred metres. These populations died-out in 1997. In 1996 the distance to the nearest *M. alcon* population appeared to be a good predictor of occurrence ($p < 0.01$).

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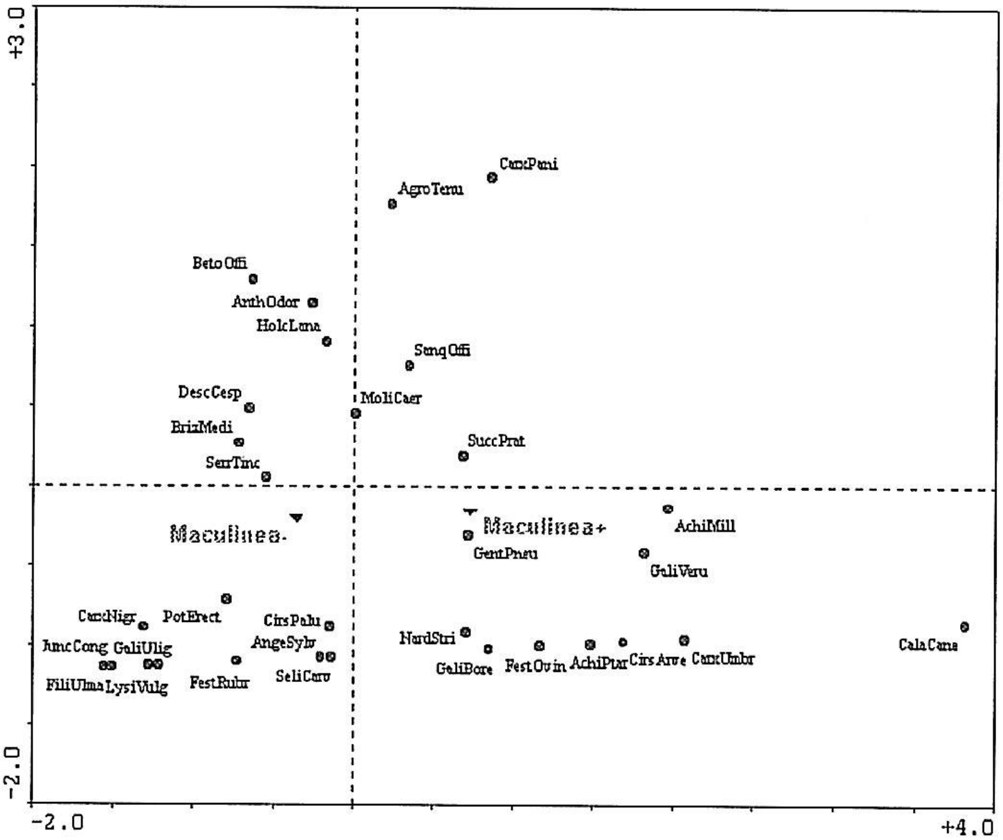

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Figure 3. CCA diagram - correlation between vegetation structure and occurrence of Maculinea alcon *populations. There are no significant differences between the vegetation structure at sites with present and* (?? ...) *absent* M. alcon. *Absence of* M. alcon *populations is gently correlated with occurrence of* Deschampsia ceaspitosa, Filipendu~~l~~la ulmaria, Angelica sylvatica, Lysimachia vulgaris *and several other species of later* = successional stages ? *succession series. Abbreviations of species names are in Appendix 2.*

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Results of statistical analysis show no significant differences in vegetation structure at sites where *Maculinea alcon* occurred or was absent (Fig. 3). není to v App. 1, jak [illegible] The population sizes of the host-plant *Gentiana pneumonanthe*, in particular the numbers of all flowering individuals at site appearred to be a very good predictor of the occurrence of *Maculinea alcon* (Table 1, Fig. 4). Most *M. alcon* populations occur at sites with several hundred gentians and seldom at sites with less than 100 gentians. We found no correlation between the gentian population types and the occurrence of *Maculinea alcon*. Two-thirds of the *M. alcon* populations occurred at sites with stable (normal) populations of *G. pneumonanthe* and the rest at sites with senile *G. pneumonanthe* populations. We found only two populations of *G. pneumonanthe* at the invasive population stage but no *M. alcon* butterflies were present there.

Table 1. Logistic regression - the population sizes (number of all flowering individuals) of the host-plant *Gentiana pneumonanthe* appear to be a very good predictor of *Maculinea alcon* occurrence. $p < 0.05$ are bold.

	Number of *M alcon* populations	**Number of *G. pneumonanthe* sites**	**logit (x)**
1996	13	31	**p < 0.001**
1997	7	31	**p < 0.010**
1998	9	43	**p < 0.005**
1999	10	50	**p < 0.001**
2000	10	52	**p < 0.001**

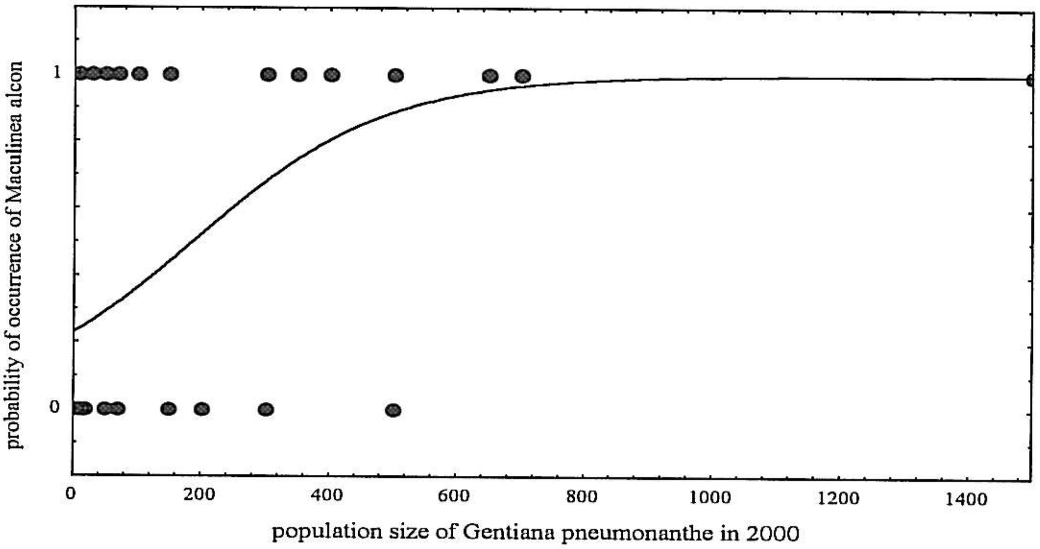

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Figure 4. Logistic regression – the population size (resp. the number of all flowering individuals) of the host-plant Gentiana pneumonanthe *is a good predictor of the occurrence* of Maculinea alcon *($p<0.001$).*

Results of logistic regressions (Table 2) showed that the probability of occurrence of *M. alcon* decreases with increasing average annual precipitation ($p < 0.001$) and number of rainy days in summer ($p < 0.005$; Appendix 1). Data for the years 1996 and 2000 show significant correlation between high average annual precipitation and *M. alcon* absence ($p < 0.01$, Fig. 5). The number of

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rainy days is significantly correlated with the absence of *M. alcon* in 2000 ($p < 0.001$). In 1996 the correlation was close to significance level ($p = 0.0546$).

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Table 2. Logistic regression – climatic conditions. Long-time averages of annual precipitation and numbers of rainy days in summer (June – July) are very good predictors of *Maculinea alcon* occurrence. Logistic regressions were count both for *M. alcon* populations known in 1996 and 2000. The third column contains results for all *M. alcon* populations known in years 1996 – 2000. p < 0.05 are bold.

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	1996	2000	1996 - 2000
	p	p	p
temperature	0.145	0.164	0.119
precipitation	**0.0096**	**0.006**	**0.0004**
days with temperature < -10°C	0.056	0.776	0.108
days with snow cover	**0.042**	0.096	**0.007**
rainy days in summer	0.0546	**0.0006**	**0.002**

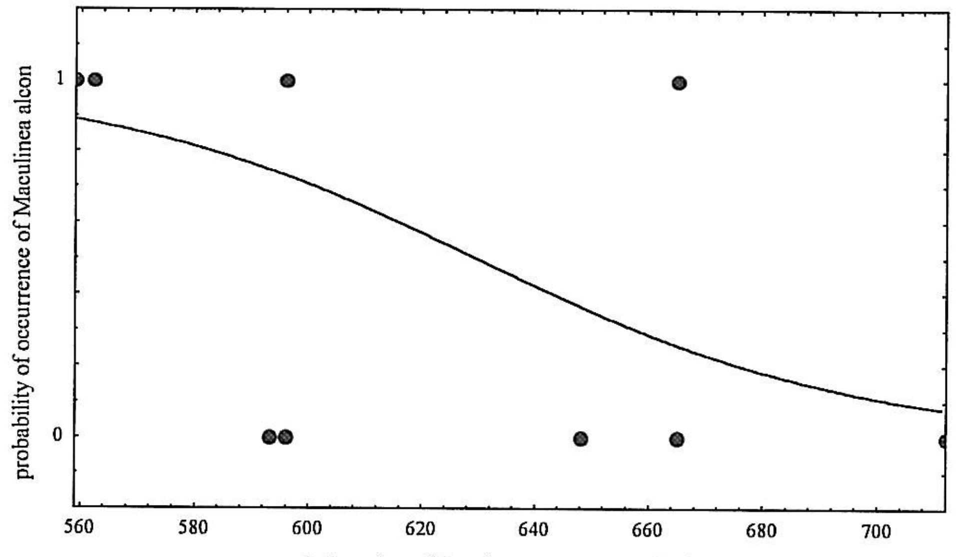

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Figure 5. Logistic regression – the average annual precipitation is a good predictor of the occurrence of Maculinea alcon *(p<0.01).*

We recorded all potential host ant species - *Myrmica rubra, M. scabrinodis*, and *M. ruginodis* together with several other *Myrmica* species (usually *M. vandeli* and *M. speciodes*) at many sites including those without *M. alcon*.

Factors causing *Maculinea alcon* extinction

We found twelve *Gentiana pneumonanthe* sites in the MTA Boletice in 1996 – 2000 (Table 3). Ten of them we found in 1996 and two more in 1999 and 2000. Their areas varied between 0.01 – 5 ha and altitude 580 – 760 m a.s.l. Distances between sites were from several hundreds meters to several kilometres. More than five hundred flowering individuals occurred in the largest population and only several individuals in the smallest population. Vegetation was in various successional sta~~t~~ges (DCA, Fig. 6). - jaká stádia jsou sukcesní a proč. ??

In 1996 we recorded the occurrence of *Maculinea alcon* at five sites. We noted the flying imagoes and many eggs on buds and flowers. In 1997 no imagoes or eggs were recorded although we checked the sites every 10 days and searched most of the gentians for eggs. We observed no change in biotic and abiotic conditions. The detailed monitoring of all these sites brought the same negative result in 1998 and 1999. We compared the biotic and abiotic characteristics from 1996 and 2000 found no important differences. There were small differences in the number of flowering gentians. Potential *Myrmica* ant-host species (*Myrmica rubra, M. scabrinodis*, and *M. ruginodis*) occurred at all the original *Maculinea alcon* sites. The most common was *M. scabrinodis* (average 56% of all *Myrmica* nests), usually between 25 – 35 nests per 75 m^2. *M. rubra* nests represented about 24% and *M. ruginodis* 13% of all *Myrmica* species nests.

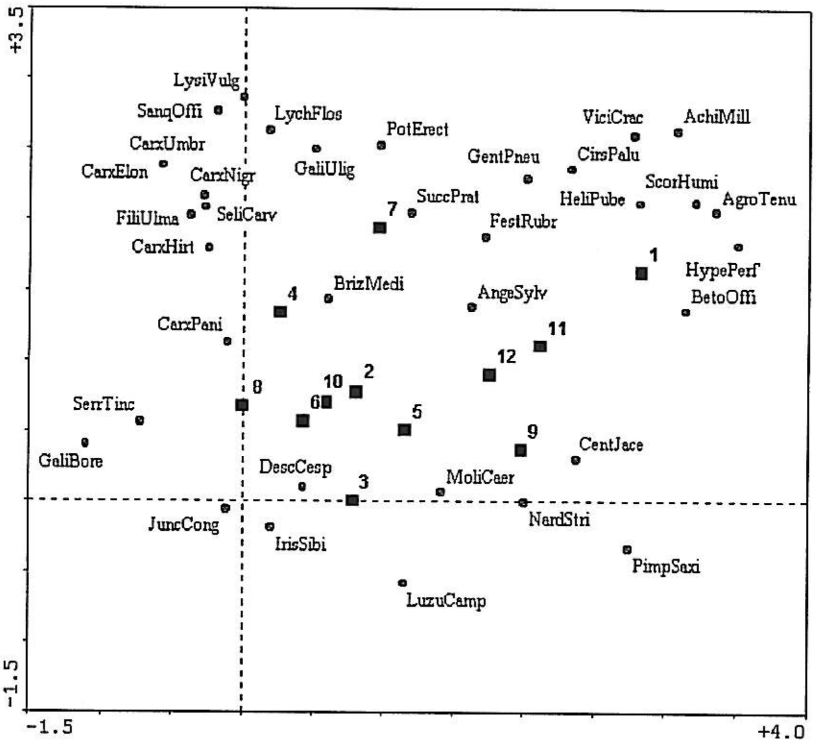

Figure 6. DCA diagram – vegetation structure at sites with occurrence Gentiana pneumonanthe *and* Maculinea alcon *populations in the Military training area Boletice.*

Numbers of sites correspond with numbers in Appendix 1. Abbreviations of species names are in Appendix 2.

Long-term climatic data shows that the MTA Boletice is wetter and colder than other parts of the study area. The average annual precipitation is about 40 mm higher, snow lays longer and the average temperature is lower there (Table 3).

Table 3. Long-term climatic data. GP – the average climatic characteristics for the all studied sites of *Gentiana pneumonanthe* in the region of study interest; MA – the average climatic characteristics for the all sites, where Maculinea alcon occurred; BO – the average climatic characteristics for the study sites in the MTA Boletice.

	GP	MA	BO
temperature [°C]	7.3	7.3	6.6
precipitation [mm]	605.1	597.4	634.7
days with temperature < -10°C	8.8	6.7	6.9
days with snow cover	58.5	56.3	71
rainy days in summer	40.3	39.4	43.7

Detailed climatic data from 1994 – 1999 (Fig. 7) indicate that a drier and colder summer in 1997 followed the very cold winter 1995/96 with deep snow cover and a wet May. Winter 1996/97 was again cold but snow cover was less than 5 cm deep. June, July and the beginning of August 1997 were extremely wet. Torrential rain occurred during the second decades of July 1997. At that time the daily precipitation reached over 40 mm and average temperature was below 15°C.

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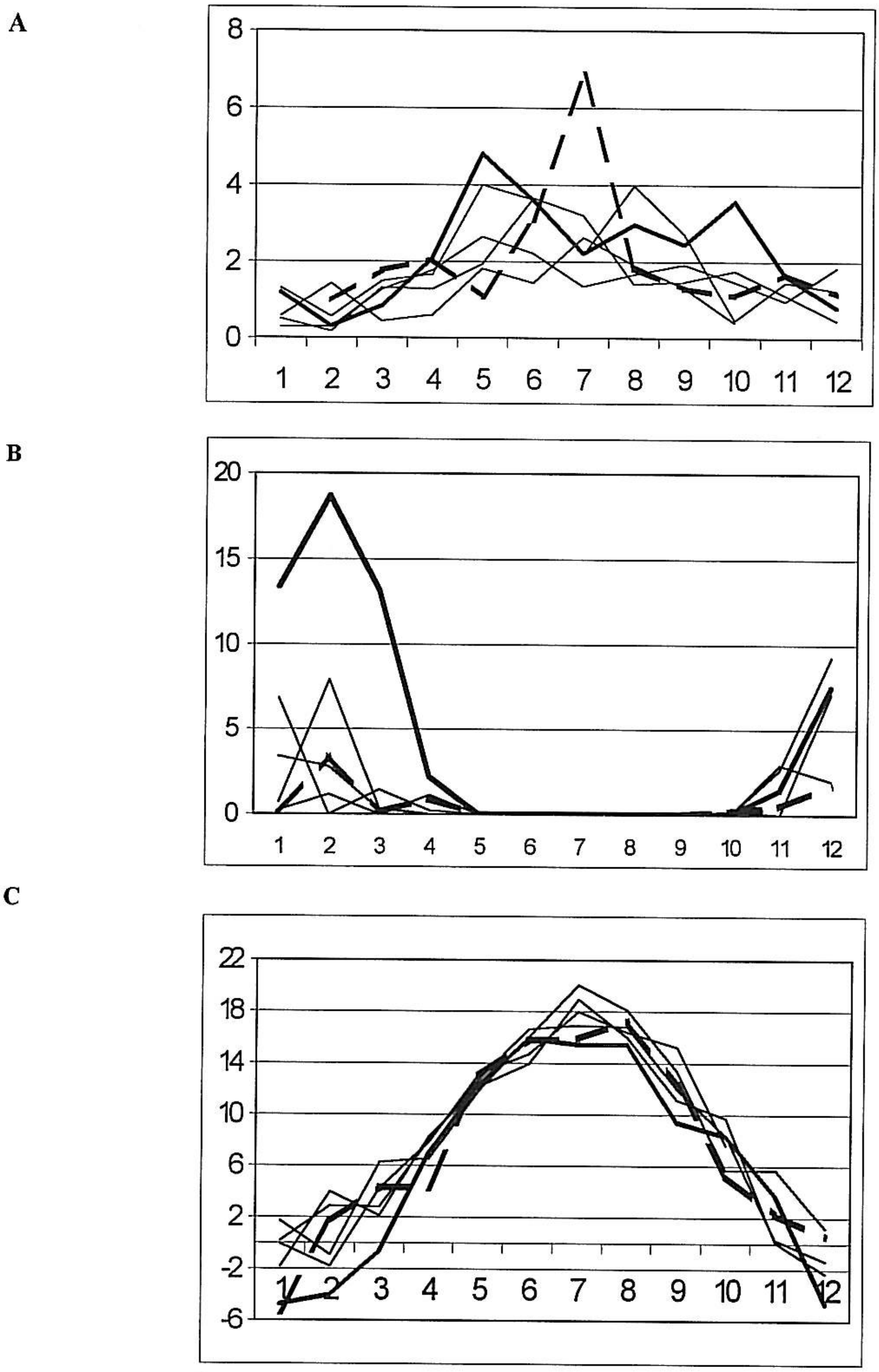

Figure 7. Climatic diagrams for sites in the Military training area Boletice for years 1994 – 99: A – precipitation [mm], B - snow cover [cm], C - temperature [C°], ▬ 1996; ▬ ▬ 1997; —— 1994, 1995, 1998, 1999.

Discussion

Defining the habitats of *Maculinea alcon* in Central Europe

During the five year project we found *Maculinea alcon* populations only at few of the site where *G. pneumonanthe* populations occurred (Appendix 1). *M. alcon* populations occur in small protected areas (Natural Reservation; usually < 5 ha), Military training areas, and regions where traditional management practices are still applied. This corresponds with information from other West European countries where most of the *M. alcon* populations became extinct during the 20th century or persisted only in isolated populations. Also in our area most populations were isolated. The data for 1996 indicated that the distance to the nearest occupied sites the distance to the nearest occupied sites is a good predictor of *M. alcon* occurrence (p < 0.01). This was not the case in subsequent years when butterflies disappeared from the sites on the MTA Boletice. Therefore this predictor is an artefact and illustrates only that *M. alcon* populations are usually aggregated in regions of high conservation status. In fact the majority of *M. alcon* populations are isolated because distances between populations are usually more than fifteen kilometres. Imagoes fly far less than 1 km so there is little dispersion between populations is very little. This means that is a probability of genes exchange (gene flow) among populations. Gene flow is one way of a sustaining the genetic diversity within a population. Populations loose genetic diversity, which means a reduction in their adaptability to environmental change in the absence of gene flow (Slatkin 1994). A genetical study of *M. alcon* populations in Denmark (Gadeberg & Boomsma 1997) showed that in several populations there were more homozygotes than would be predicted if random mating occurred. Also the population genetics study on *M. nausithous* and *M. teleius* confirmed that genetic variability is significantly lower in isolated populations (Figurny-Puchalska et al. 2000).

Effect of habitat fragmentation on *M. alcon* was studied in the Netherlands (Wynhoff et al. 1996), where all populations in moist hay-meadows have became extinct and heathlands populations are isolated and in a habitat of poor quality. Rapid extinction of populations occurred in the province of Drenthe, where nine populations where recorded in 1981-85, two of which became extinct in 1986-90 and three more in 1991-95. A similar result was recorded also in the other West European countries. Unfortunately there are no long term data on the occurrence and extinction of *M. alcon* in the Czech Republic and other Central European countries, but similar trends are likely to have occurred. We recorded several extinctions during our five year project (Appendix 1).

Gentiana pneumonathe is the unique host-plant for larvae of *M. alcon* and the population size of the food-plant appeared to be a very good predictor of *M. alcon* occurrence (p<0.001). Climatic conditions and management practices caused slight yearly change in numbers of flowering individuals at many sites. The ability of gentians to remain in dormant for one or more years (Oostermeijer et al. 1994) also caused fluctuations. We found few butterflies at sites with low numbers (< 100) of flowering gentians. Usually these sites were close to larger populations or in areas where originally large populations were fragmented into several smaller ones. No significant correlation was found between *G. pneumonanthe* population types and the occurrence of butterflies. *M. alcon* populations occurred both at sites with stable and senile populations of its host-plant. Senile populations, with only adult gentians, are suitable for *M. alcon* butterflies because juvenile plants are not suitable host-plants and their absence doesn't influence the suitability of the sites. Although marsh gentians are long-aged, the vitality and demographic structure of *G. pneumonanthe* populations are important for the long term survival of *M. alcon* population. Time and intensity of disturbances of sites affect the demographic structure of *G. pneumonanthe* populations, because the marsh gentian only reproduce by seed and several authors (Oostermeijer et al. 1994, Křenová & Lepš 1996, Kesel & Urban 1999) have confirmed that places with bare soil (gaps) are essential for the successful regeneration of *G. pneumonanthe*. Small-scale gaps are more common in traditionally managed oligotrophic wet meadows and short grass pastures but stronger disturbances and larger gaps are needed in meadows abandoned for a long time (Křenová 2001). Time from abandonment and management practices affect the demographic structure of the host-plant population and the species composition and vegetation structure at these sites. Although the statistical analysis showed no significant differences in vegetation structure at sites with and without *M. alcon*, the absence of *M. alcon* was weakly correlated with species that are typical of late succession in wet oligotrophic meadows. In long abandoned meadows the tall grasses (*Molinia coerulea, Deschampsia ceaspitosa*) and wide-leaves plants (*Filipendulla ulmaria, Angelica sylvatica, Lysimachia vulgaris* etc.) are dominant, and vegetation is dense and the litter layer thick. The gentians are usually tall and have many stems and flowers but the effective population size (here defined as the number of gentians oviposited on) rapidly decreases.

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G. pneumonanthe and *M. alcon* occur in various habitats in addition to very wet meadows with a high cover of mosses, periodically dry meadows with clay soil or rocky pastures with a variable underground water level.

Local biotic and abiotic conditions affect species composition and abundance of *Myrmica* ants in various habitats (Clarke et al. 1998). Our results correspond with those from other pars of Europe where vegetation structure and soil humidity limit occurrence of *Myrmica* colonies. In our study area we found all the three potential host *Myrmica* ant species (*M. rubra, M. scabrinodis*, and *M. ruginodis*) at many sites. At five sites we found larvae of *M. alcon* in nests of *M. scabrinodis*, which indicate that *M. scabrinodis* is the primary host species in our study area. This observation adds information about the different host ants in different parts of Europe (Elmes et al. 1998).

The analyses of climatic data indicate that both average annual precipitation and number of rainy days in summer are very good predictors for *M. alcon* occurrence. In addition the number of days with snow cover also appeared to be a good predictor. All these factors were negatively correlated with the occurrence of *M. alcon*. *M. alcon* butterflies are absent from the colder parts of the region.

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Factors causing *Maculinea alcon* extinction

The appearance of several new large *G. pneumonanthe* and *M. alcon* populations in the MTA Boletice in 1996 followed by the extinction of the butterflies focused our interest on the factors causing extinction. In 1997 and 1998 we speculated that caterpillars' may stay in ants' nests for longer than a year, as was known for *M. rebeli* (Minguira - personal communication). Later the occurrences of both annual and biennial life cycles was recorded in *M. alcon* populations (Schönrogge et al. 2000). They recorded that larvae of *M. alcon* excavated from ants' nests at the pre-pupation time, at six sites, showed two distinct weight cases and the body weight distribution was bimodial. The elongation of development beyond two years is unlikely. The probability that *M. alcon* at the MTA Boletice area is extinct is very high because no *M. alcon* imagoes or eggs were observed for four years. We tried to find the factors that caused the extinction. No significant differences were found in vegetation structure, total percentage cover or in the percentage cover of bryophytes in years before and after extinction. Small annual fluctuations were recorded in the numbers of flowering gentians but the numbers were sufficient at origin *Maculinea alcon* sites. Because we did not know which *Myrmica* species hosted *M. alcon* we investigated the occurrence of *Myrmica rubra, M. scabrinodis*, and *M. ruginodis*. All three species occurred at the origin *M. alcon* sites and their abundance and distribution of colonies were similar to the to situation at other *M. alcon* sites of similar habitat quality. Dominance of M. *scabrinodis,* which is more termophilic than *M. rubra* and *M. ruginodis* and tolerant of high soil humidity (Seifert 1988), corresponds with the wet meadow habitats of *G. pneumonanthe* in the MTA Boletice. The density of 25-35 nest/75m^2 is slightly higher than is recorded from south of England (Elmes & Wardlaw 1982 a, b; 25 nests/100m^2) and the south of the former East Germany (Seifert 1988; 19.1 nests/100m^2).

= classes ?

Long-term climatic data showed that the MTA Boletice is colder and has a high annual precipitation and long snow cover (Table 4). Detailed climatic data from 1994 – 1999 (Fig. 8) showed that 1996 and 1997 were colder with extremely wet summers. These conditions caused not only high a mortality of ant brood but also affected the vitality and survival of *Maculinea* larvae (Thomas et al. 1993). In the summer of 1997 *M. alcon* populations were probably stressed by extremely bad weather during the flight period. It is likely these factors could cause the extinction in sites on the edge the butterfly's climatic range. High probability of extinction at the edge of a species range is recorded for *Maculinea arion* (Thomas et al. 1998b).

| [illegible]

Originally immigrants from populations in the centre of the distribution probably maintained *M. alcon* populations on the edge of their range. Recently because of habitat is fragmentation most sites have been isolated from each other, and the probability of natural reintroduction is now close to zero. All the historical sites for *G. pneumonanthe* close to the MTA were drained and disappeared several decades ago. Migration from the south is unlikely because the Lipno dam was built in the 1950s and destroyed the natural migration corridor for many species. Migration from the north is also unlikely because the nearest *M. alcon* population is more than 30 km away. The residual populations with only a few flowering individuals are at distances of more than 20 km. We conclude that there is no chance of natural reintroduction of the Blue alcon butterfly to sites in the region where the conditions are suitable for the species because of habitat fragmentation.

Implication for Conservation

Maculinea alcon is one of the most endangered species in the European entomofauna. The life cycle of *Maculinea* butterflies is fascinating and extremely vulnerable. It is unlikely that caterpillars stay in ant nests for more than two years. Therefore, most recent populations are doomed to extinction. In landscapes, with fragmented habitats species that can only fly for short distances can not recolonise empty sites. The species can survive only in regions where functional metapopulations still survive. For the successful development of these butterflies various disturbances to the vegetational structure are essential. Natural disturbances - several days of flooding, short time burning and impact of big animals (deer, boars) are important in changing the balance of the demographic structure of the host-plant populations. Also the spatial dynamics of ant nests is positively affected by these factors. Human activities, the hand mowing for 3 – 5 (7) years and grazing by cows, sheep or horses are important for the survival of *M. alcon* populations. Mowing by machinery is insufficient because ants' nests are often destroyed by this process and hay meadows are often mown in July when *Maculinea* females oviposit on gentian buds. Results from the MTAs and other sites showed that strong disturbances of parts of a site can disrupt successional changes in long abandoned meadows, rejuvenate the host plant populations and ensure suitable habitats for several next decades.

Acknowledgements

The research was supported by the Grant Agency of the Czech Academy of Sciences (junior grant GA AV ČR 6141901) and the Mattoni Award. We would like to thank the managers of the Military training area Boletice for possibility to perform our research in the MTA. We are grateful also to many local managers, amateur botanists and lepidopterists, and conservation authorities, who inform us about the gentians and butterflies populations. We are grateful to Jan Lepš and Martin Konvička for his comments and to Anthony F. G. Dixon for correcting English.

English!

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Appendix 1

Biotic and abiotic characteristics of 52 study sites.

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* - *populations of* Gentiana pneumonanthe *extinct during study period 1994 – 99 and were not included in analysis;* ^{B} *– sites in the Military training area Boletice, the numbers correspond with numbers in Figures 6 and 7;* ***Maculinea:*** *M94 – M99 – occurrence/absence (1/0) of* M. alcon *populations at sites in years 1994 – 1999;* ***Gentiana:*** *GP99 – number of flowering individuals of* G. pneumonanthe *in 1999; Poptype – type of* G. pneumonanthe *population, 1 – invasive, 2 – stabile, 3 – senile; Height – average height of stems [cm]; Stem – average number of stems per one individual; Flower - average number of flowers per one individual;* ***Vegetation:*** *E% - total % cover; Eo% - % cover of mosses;* ***Site:*** *Area - site area [ha]; AL – altitude [m a.s.l.]; DS – average distance to three nearest sites [km]; NR – (1/0) the site is part of the Nature reservation; MG – management, 1 – grazing, 2- hand mowing, 3 – machine mowing, 4 – mechanical irregular disturbances, 5 – unmanaged sites;* ***Climate:*** *T – the average annual temperature, P – annual precipitation, F – number of days with temperature < -10°C, S – number of days with snow cover, R – number of rainy days in summer (Juni – August).*

	Maculinea						Gentiana					Vegetation		Site					Climate				
Site No.	M94	M95	M96	M97	M98	M99	GP99	Poptype	Height	Stem	Flower	E%	Eo%	Area	AL	DS	NR	MG	T	P	F	S	R
1	0	0	0	0	0	0	12	3	36	2.7	1.7	90	10	0.1	540	15	1	5	8.1	579	14	47	40
2	.	.	0	0	0	0	10	3	53.9	3	3.5	95	5	0.4	430	18	1	5	8.1	579	14	47	40
3*	.	.	0	.	.	0	0																
4	.	.	0	0	0	0	1	3	48	13	8	85	20	0.1	430	18.2	1	5	8.1	579	14	47	40
5	.	.	0	0	0	0	1	3	48	12	9	70	20	0.02	420	21.5	1	5	8.1	579	14	47	40
6*	.	.	0	.	.	0	0																
7	.	.	1	1	1	1	1400	2	38.1	2.5	15.2	95	5	2.4	550	7	0	2	8.4	572	15	46	28
8	1	1	1	1	1	1	300	2	46.5	2.3	15.3	95	5	1	500	5	0	2	7.4	665	4.3	57	45
9	0	0	0	0	0	0	7	3	58.8	3.7	21.2	95	15	0.75	510	4.8	1	5	7.4	665	5.2	57	45
10	1	1	1	0	0	0	10	2	44.9	2.7	7.2	90	10	1	500	5	0	6	7.4	665	5.2	57	45
11	0	0	0	0	0	0	7	3	38.1	2.4	5.6	95	2	0.01	410	22.3	1	6	7.2	596	7.3	51	39
12	.	.	1	1	1	1	50	3	52.9	3.2	24	98	1	1.2	430	24.6	0	6	7.2	596	7.3	51	39
13*	.	.	0	.	.	0	0																
14	.	1	1	1	1	1	600	2	39.5	3.3	13	98	4	2	480	12.3	0	3	7.1	562	6.6	60	39
15	.	.	1	1	1	1	150	2	55.7	3.5	23.1	98	5	0.8	500	7	1	5	7.1	559	6.6	59	39
16*	.	0	0	.	.	0	0																
17	.	.	.	.	0	0	150	3	36.6	3.6	28.4	85	10	0.5	460	5.9	1	2	7.7	611	15	46	32
18	.	.	1	1	1	1	200	2	34.5	2.5	6.9	97	15	1	510	6	1	3	7.1	559	6.6	59	39
19	1	1	1	1	1	1	450	2	35.9	2.7	5.1	75	40	3	500	43.8	1	3	7.5	710	13	41	36
20	.	.	0	0	0	0	300	2	45.3	3.7	8.8	85	50	1	400	12.8	1	3	7.4	593	5.2	45	41

Appendix 1 Continued.

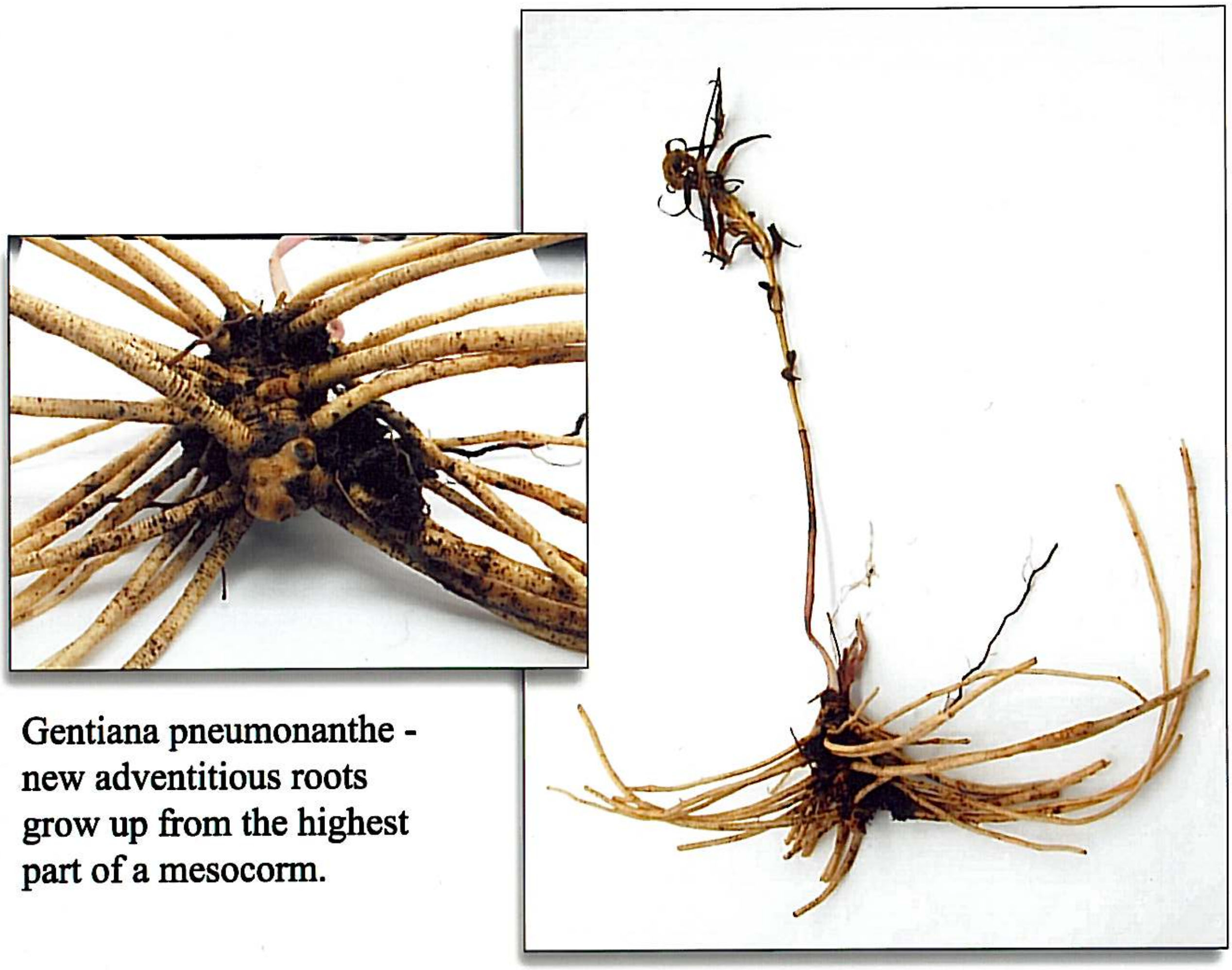
			Maculinea						Gentiana				Vegetation				Site				Clima		
Site No.	M94	M95	M96	M97	M98	M99	GP99	Poptype	Height	Stem	Flower	E%	Eo%	Area	AL	DS	NR	MG	T	P	F	S	R
21^{B1}	.	.	1	0	0	0	150	2	39.4	2.7	9.6	98	3	5	620	5	0	4	7	596	4.3	61	43
22^{B2}	.	.	1	0	0	0	150	2	46.6	2.2	11.9	90	7	1.5	580	1.3	0	2	7	596	4.3	61	43
23^{B3}	.	.	1	0	0	0	20	3	49.7	1.6	6.1	95	0	1	580	1.6	0	4	7	596	4.3	61	43
24^{B4}	.	.	1	0	0	0	450	2	53.9	3.6	16.5	90	10	1	570	1.9	0	4	7	596	4.3	61	43
25	.	.	0	0	.	0	6	3	54	4.1	23.3	95	15	0.5	680	5.6	1	1	7.4	648	5.2	72	47
26	.	.	0	0	0	0	1	3	48	2	7	95	5	1	650	5.5	0	4	7.4	648	5.2	72	47
27	.	.	0	0	0	0	1	3	60	1	8	85	1	0.75	610	9.7	0	2	7.4	648	5.2	72	47
28^{B8}	.	.	1	0	0	0	550	2	37.3	1.8	5.5	90	10	7	600	1.6	1	4	7	596	4.3	61	43
29^{B9}	.	.	0	0	0	0	5	3	39	5.5	15.5	95	20	0.5	630	1.25	1	4	7	596	4.3	61	43
30^{B5}	.	.	0	0	0	0	100	2	54.3	3.7	9.9	95	5	1	740	3.6	1	2	5.9	712	12	91	45
31^{B6}	.	.	0	0	0	0	3	3	32.5	4.5	5	98	4	0.01	760	2.9	1	5	5.9	712	12	91	45
32^{B7}	.	.	0	0	0	0	40	2	37	4	6	95	5	0.4	800	3	1	4	5.9	712	12	91	45
33	.	.	1	1	1	1	100	2	13.1	2.9	4.7	85	10	2	670	25	1	1	6	691	8.4	82	47
34	.	.	.	.	0	0	43	1	34.8	1.5	4.1	70	50	0.05	720	23.8	1	4	8.1	579	14	47	40
35	.	.	.	.	0	0	8	3	46.4	2.8	10	85	10	0.01	650	35	1	5	7.1	581	6.6	60	40
36	.	.	.	.	0	0	4	3	46.8	4.6	31.2	95	3	0.05	650	32.1	1	5	7.1	581	6.6	60	40
37	.	.	.	.	1	1	450	2	32.5	2	14.5	85	0.5	5	480	10.5	1	2	7.1	559	6.6	60	39
38	.	.	.	.	0	0	300	2	46.4	2.4	23.6	90	5	3	500	5.8	1	2	7.7	611	15	46	32
39	.	.	.	.	0	0	1	3	72	9	33	95	7	3	500	5.6	1	5	7.7	611	15	46	32
40	.	.	.	.	0	0	15	3	49.1	2.7	22.9	97	3	0.01	650	22.3	1	5	6	691	8.4	72	43
41	.	.	.	.	0	0	80	3	30.1	4.9	2.1	90	20	0.05	560	32	0	5	8.4	572	15	46	28
42	.	.	.	.	0	0	700	2	35.9	2.4	8.9	98	50	2	560	30.5	1	2	8.4	518	13	31	37
43	.	.	.	.	1	1	500	3	44.4	3.6	42.4	98	2	2	440	7.1	1	5	7.1	559	6.6	59	39
44	.	.	.	.	.	0	5	3	42	2.6	0.2	75	0.5	0.2	580	9.5	1	5	8.4	572	12	46	28
45	.	.	.	.	.	1	70	3	52	2.1	9	90	5	1	510	6.3	1	5	8.4	572	12	46	28
46	.	.	.	.	.	0	20	3	44.2	5.4	14	95	5	1	500	16.5	1	5	7	548	15	72	40
47	.	.	.	.	.	0	70	3	53.1	6.1	17.2	97	20	2	500	16.5	1	5	7	548	15	72	40
48	.	.	.	.	.	0	60	3	56	3	15.2	98	15	3	500	28.3	1	5	7	596	4.3	61	43
49*^{B10}	.	.	.	.	0	0	2																
50^{B11}	.	.	.	.	.	0	6	3	37	2	21.8	98	2	0.2	580	4.7	1	5	7	596	4.3	61	43
51	.	.	.	.	.	0	3	3	20.3	4.7	3.6	95	5	0.1	560	31	0	5	8.4	518	13	31	37
52^{B12}	.	.	.	.	.	0	50	1	22.3	3.1	6.1	85	2	3	620	5	0	4	7	596	4.3	61	43

Appendix 2. List of species in figures of CCA and DCA analysis.

Nomenclature: Rothmaler (1976).

AgroTenu	*Agrostis tenuis*
AchiMill	*Achillea millefolium*
AchiPtar	*Achillea ptarmica*
AngeSylv	*Angelica sylvestris*
AnthOdor	*Anthoxanthum odoratum*
BetoOffi	*Betonica officinalis*
BrizMedi	*Briza media*
CalaCane	*Calamagrostis canescens*
CarxElon	*Carex elongata*
CarxHirt	*Carex hirta*
CarxNigr	*Carex nigra*
CarxPani	*Carex panicea*
CarxUmbr	*Carex umbrosa*
CentJace	*Centaurea jacea*
CirsArve	*Cirsium arvense*
CirsPalu	*Cirsium palustre*
DescCesp	*Deschampsia ceaspitosa*
FesrOvin	*Festuca ovina*
FestRubr	*Festuca rubra*
FiliUlma	*Filipendula ulmaria*
GaliBore	*Galium boreale*
GaliUlig	*Galium uliginosum*
GaliVeru	*Galium verum*
GentPneu	*Gentiana pneumonanthe*
HolcLana	*Holcus lanatus*
HypePerf	*Hypericum perforatum*
IrisSibi	*Iris sibirica*
JuncCong	*Juncus conglomeratus*
LuzuCamp	*Luzula campestris*
LychFlos	*Lychnis flos-cuculi*
LysiVulg	*Lysimachia vulgaris*
MoliCaer	*Molinia caerulea*
NardStri	*Nardus stricta*
PimpSaxi	*Pimpinella saxifraga*
PoteErec	*Potentilla erecta*
SanqOffi	*Sanquisorba officinalis*
ScorHumi	*Scorsonera humilis*
SeliCarv	*Selinum carvifolia*
SerrTinc	*Serratula tinctoria*
SuccPrat	*Succisa pratensis*
ViciCrac	*Vicia cracca*

Gentiana
pneumonanthe -
morphological
plasticity



Gentiana pneumonanthe -
new adventitious roots
grow up from the highest
part of a mesocorm.

Two years old individual in a gap.

VAM mycorhiza.

Pollinators of Gentiana pneumonanthe

Maculinea alcon