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Forest ground beetle assemblages and population genetics in the Wellin district (Ardennes, Belgium): a forest historical approach

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Abstract

Within the context of a detailed study on the effects of the predatory checkered beetle *Thanasimus formicarius* on Scolytid beetle populations, three forest sites were sampled by means of pitfall traps in the Wellin forest district (Walloon region, Belgium) during 2002. The sites included a mixed pine forest (Chanly), a spruce plantation (Wellin) and a mixed ancient deciduous forest (Neupont). At Neupont and Chanly, additional population genetic samples were obtained for two forest carabid species and analysed through allozyme electrophoresis. Forest history of the area was documented in detail by old maps from 1775 onwards.

Ground beetle assemblages mainly differed between the spruce forest at Wellin and the other two sites, which were dominated by specialized forest species with a lower dispersal power. At Wellin, a site heavily perturbed by wild boar and situated close to open habitat, several generalist ground beetle species were encountered, indicative for more disturbed and/or open forest sites. Analysis of forest history revealed that part of these differences coincides with a different age of continuous afforestation on the three sites and their immediate surroundings.

Parallel population genetic studies on two forest carabid species (*Abax ater* and *Carabus problematicus*) showed, compared to previous results from other forests in Belgium, high values of genetic diversity in the ancient deciduous forest plot. This is understood as a consequence of the large forest surfaces, which have been continuously present in this region. A significantly lower genetic diversity was observed for both species in the more recent mixed forest site. *Carabus problematicus* even showed a considerable loss in alleles at three independent loci. This could have resulted from a relatively recent colonization history of a restricted number of individuals or, alternatively, from local adaptation phenomena. Whether these differences are purely neutral and/or adaptive will be investigated in future studies.

We conclude that, in this area, typical forest ground beetles have been able to colonize and establish themselves, at least in low numbers, on sites that were not forested about 250 years ago. Our genetic results, however, warn that such recent forest populations are genetically impoverished. Forest history as well as ecology and management therefore seem to have an impact not only on observed ground beetle species, but also on observed intraspecific genetic diversity levels. Both sets of factors will be evaluated in the future on a much larger dataset for different eco-regions in our country.

Key words: Carabidae, ecology, forest history, ground beetle assemblages, population genetics, genetic diversity, genetic differentiation, ancient forest, recent forest

Résumé

Durant l'année 2002, lors d'une étude détaillée portant sur les effets de la prédation du Cléride *Thanasimus formicarius* sur les populations de coléoptères Scolytides, trois sites forestiers ont été échantillonnés à l'aide de pièges à fosses dans le cantonnement forestier de Wellin (Wallonie, Belgique). Les sites consistaient en une forêt mixte de pins (Chanly), une plantation d'épicéas (Wellin) et une ancienne forêt mixte de feuillus (Neupont). A Neupont et Chanly, des échantillons supplémentaires pour des études génétiques de populations ont été prélevés pour deux espèces de carabes forestiers et ont été analysées à l'aide d'électrophorèses d'allozymes. L'historique forestier de cette région a également été documenté en détails par d'anciennes cartes depuis 1775.

Les assemblages de carabes diffèrent principalement entre la forêt d'épicéas de Wellin et les deux autres sites, qui sont dominés par plus d'espèces forestières spécialisées, avec un faible pouvoir de dispersion. Le site de Wellin, très perturbé par des sangliers sauvages et proche d'habitats ouverts, comporte quant à lui de nombreuses espèces de carabes plus généralistes, indicatrices de sites forestiers plus perturbés et/ou plus ouverts. L'analyse de l'historique forestier a révélé qu'une partie de ces différences coïncide avec un âge différent de boisement continu sur les trois sites et leurs environnements immédiats.

Des études parallèles de génétique de populations sur deux espèces de carabes forestiers (*Abax ater* et *Carabus problematicus*) ont montré, par rapport à des résultats antérieurs obtenus pour d'autres forêts en Belgique, de grandes valeurs de diversité génétique dans l'ancien site forestier de feuillus situé à Neupont. Ceci peut s'expliquer comme une conséquence des larges surfaces forestières qui ont été continuellement présentes dans l'histoire de cette région. Une diversité génétique significativement plus faible a été observée pour les deux espèces dans le site forestier mixte plus récent. *Carabus problematicus* a même montré un manque considérable d'allèles sur trois loci indépendants qui pourrait résulter d'une colonisation relativement récente d'un nombre restreint d'individus ou, alternativement, de phénomènes locaux d'adaptation. Déterminer si ces différences sont purement neutres et/ou adaptatives fera l'objet d'études futures.

Nous concluons que, dans cette région, des carabes typiquement forestiers ont pu coloniser et s'établir au moins en petits nombres, sur les sites, qui n'étaient pas boisés il y a 250 ans. Cependant, les résultats génétiques obtenus attirent notre attention sur le fait que des populations forestières si récentes sont génétiquement appauvries. L'historique forestier aussi bien que l'écologie et la gestion forestière semblent donc avoir un impact non seulement sur les espèces de carabes observées, mais aussi sur les niveaux de diversité génétique intraspécifique observés. Ces deux facteurs seront évalués dans le futur sur des ensembles de données beaucoup plus étendus pour différentes éco-régions de notre pays.

Mots clés: Carabidae, écologie, historique forestier, assemblages de coléoptères carabiques, génétique de population, diversité génétique, différenciation génétique, forêts anciennes, forêts récentes

Introduction

Especially in Western Europe, the amount of remaining ancient forests has reached dramatic proportions. In Belgium, as in many other parts of Europe, forests are the result of about 7000 years of human influences. Their history in general is one of woodland destruction, fragmentation and degradation, although locally (e.g. in Flanders between y1300-1800) there were also periods of forest rehabilitation and expansion. Around y1850-1895, woodland area reached its absolute minimum in our country, although there were some differences between eco-regions (cf. TACK *et al.*, 1993; TALLIER, 2004). From that period onwards, mainly in the Ardennes and the Campine region, reforestation proceeded, but principally by biologically poorer pine and spruce forests at the expense of broad-leaves stands, which further decreased until today. The decrease of ancient forests has thus been accompanied by a serious decline in forest habitat quality. Forests nowadays cover about 20 % of Belgium, but there are large differences between regions, with less than 10% of area forested in Flanders, but more than 30 % in Wallonia (HERMY *et al.*, 2003). The proportion of these forests that can be defined as 'ancient' (meaning that the past 230 years, since the first systematic maps of de Ferraris, y1770-1778, these sites have always remained forest) is even more reduced.

Recent developments in forest research have stressed the importance of historical ecology in shaping the diversity and evolutionary potential of woodland plants and animals, especially in Western Europe (VERA, 2000; HONNAY *et al.*, 2005). Numerous studies point to qualitative differences in species composition between ancient and recent forest (on plants, cf. GODEFROID & KOEDAM, 2003a,b; GRAAE *et al.*, 2003; HERMY *et al.*, 1999; on invertebrates, cf. ASSMANN, 1999; DESENDER *et al.*, 1999). These differences are supposed to be attributed at least partly to the limited colonization capacity of many species characteristic for ancient forest. Preliminary results strongly suggest that forest history is of great importance for the ecological and genetic constitution of ancient forest beetles, and thus, in the long run, for their micro- and macro-evolution (ASSMANN, 1999; DESENDER *et al.*, 1999, 2004).

Invertebrates, i.e. ground beetles, combine a number of features of high interest for such studies and, without doubt, are the most diverse component of woodlands. Ground beetles (Coleoptera, Carabidae) mostly show a high species richness and many species have a pronounced habitat preference for forest interiors. These beetles are very well documented in Belgium for what concerns their recent and historical occurrence, as can be derived from a large amount of distribution data since about 1850 (DESENDER, 1986a,b; DESENDER *et al.*, 1994), as well as from archaeological data (DESENDER *et al.*, 1999; ERVYNCK *et al.*, 1994). Most stenotopic woodland carabid species are constantly wingless or never develop functional flight musculature (ASSMANN, 1999; DESENDER, 1989; DESENDER *et al.*, 1999).

We recently started a research project in order to gain more insight into the tempo and mode of evolution of recent towards ancient forest (Belspo-project 2005: MO/36/14: How does forest history influence invertebrate diversity and evolution?). The main aim of this study is to integrate woodland history, ground beetle distribution and evolutionary genetics at differing geographical scales in Belgium. First, we will, at the national and eco-regional level, review and meta-analyse all available quantitative data on forest ground beetles from ancient and recent forests in Belgium. Secondly, we will concentrate on morphology, genetics and (micro)-evolution of three model carabid species. Finally, this project will concentrate on possible influences of forest history on a local scale. Within this last context, we also want to investigate ecology and genetics of ground beetles from additional sites in the Walloon region. The present paper fits into this topic.

Within the context of a detailed study on Scolytid beetle populations and their predator *Thanasimus formicarius* (L.) (Coleoptera, Cleridae) (WARZÉE, 2005), three different forest types were sampled in 2002 by means of pitfall traps and window traps in the Wellin forest district (Ardennes, Walloon region, Belgium). Ground beetles from these samplings were at our disposal for study. The sites included a mixed pine forest (Chanly), a spruce plantation (Wellin) and a mixed deciduous forest (Neupont). At Neupont and Chanly, additional population genetic samples were obtained for two forest carabid species and analysed through allozyme electrophoresis. At present, the study sites are part of a very large more or less continuously forested area (see further). Forest history of the area could be documented in detail by old maps from 1775 onwards.

Below, we will describe the observed ground beetle assemblages from these study sites (pitfall traps), their dispersal power as well as results on the genetic diversity and differentiation of two species, within an ecological as well as historical context. Data on ground beetle flight activity, derived from window trap catches, will be given in another paper (DESENDER *et al.*, in prep.).

Material and Methods

The three investigated forest stands are situated in the forest district of Wellin (UTM 10x10km: FR54) (Walloon region, Belgium). Fig. 1 situates the study area, at the northern fringes of the Ardennes, on a map with the actual cover of forests in Belgium. Early March 2002, three pitfall traps (glass jam jars, 9.5 cm diameter) were installed in each of the sites, along with five window traps. Details of location and characteristics of the sampling sites are summarized in Table 1. In addition to the tree level, the Chanly site showed also a well-developed shrub level, mainly composed of *Quercus* spp. and therefore it was classified as mixed pine forest. Pitfall traps were inspected and changed at weekly intervals till the end of October 2002. Especially in the spruce forest at

Table 1 — Characteristics of the sampling sites (pedology codes refer to: G= texture: sandy-loam with more than 5% stones; b= well-drained; b= soil profile: brown soil; r= schist or q= sandstone; 2= depth of soil between 40 and 80 cm)

Stand	**Stand location**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**Slope**	**Pedology**	**Area (ha)**	**Tree composition**	**Plantation date**
Pine (mixed forest)	Chanly	50° 04' 02"	05° 09' 41"	325	5-15%	G b b r 2	31.6	*Pinus sylvestris* L. (97%) open space (2%) *Pinus nigra* subsp. *laricio* Maire (1%) sub-level : *Quercus* spp.	1924
Spruce	Wellin	50° 03' 33"	05° 07' 29"	340	5-15%	G b b r 2	16.8	*Picea abies* (L.) Karst. (95%) open space (5%)	1946
Broad-leaves	Neupont	50° 02' 34"	05° 07' 55"	290	25-45%	G b b q 2	57.9	*Quercus* spp. (79%) *Fagus sylvatica* L. (20%) *Carpinus betulus* L. (1%)	?

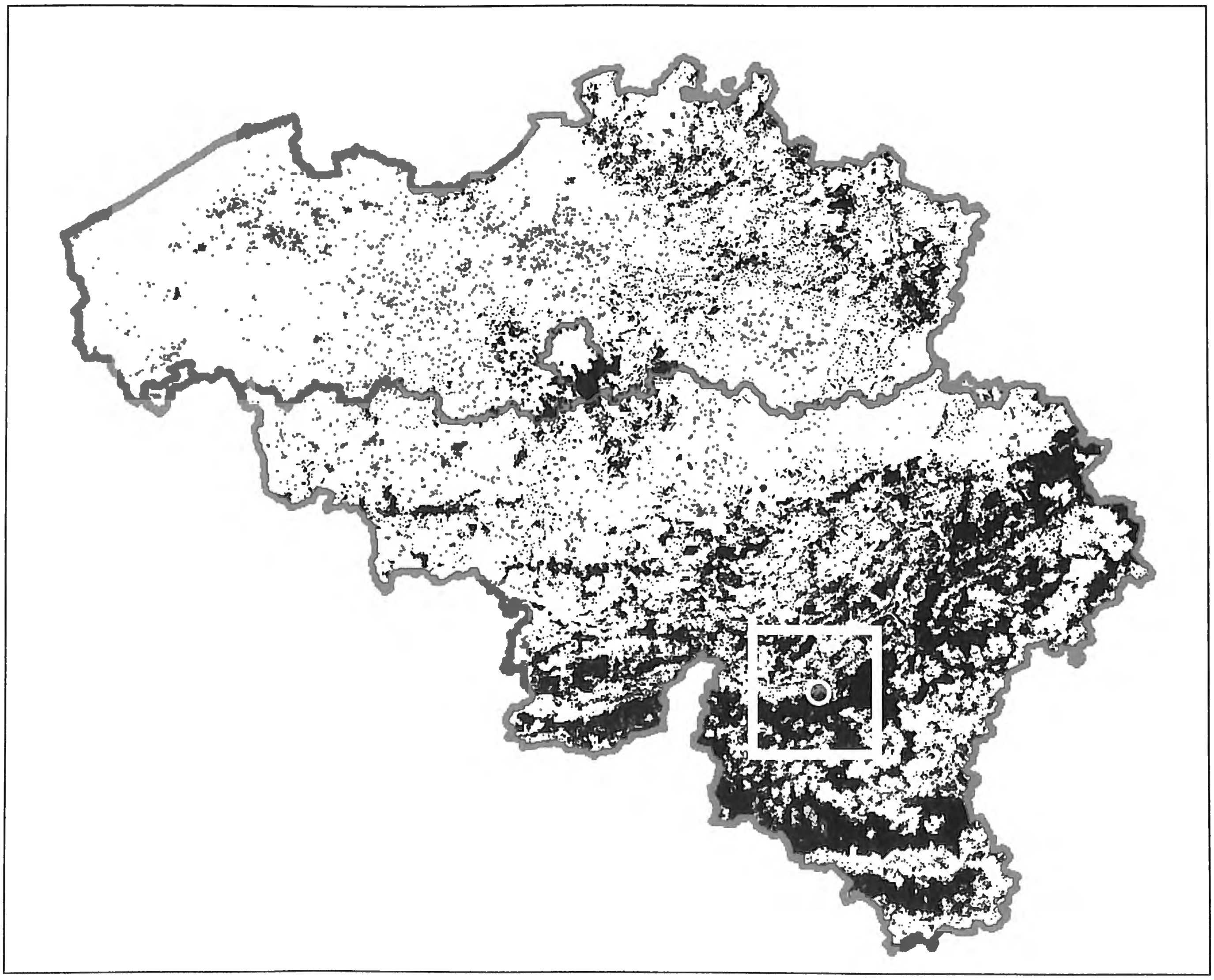

Fig. 1 — Location of the study area (white square) at the northern limit of the Ardennes on a forest map of Belgium (actual forests shown in black; regional borders added).

Wellin, wild boar damage led to trap losses at the onset of our samplings. Therefore, from June onwards, we installed protective vials with the chemical repellent cornitol around the traps. Relatively high subsequent catches of beetles suggest there was no negative influence of this repellent to trap yields of ground beetles, whereas the system proved very effective to avoid wild boar damage and disturbance to the traps. All ground beetles were identified to species level, sexed and checked for their state of wing development (macropterous or full-winged versus brachypterous or short-winged beetles).

Detrended Correspondence Analysis (DCA) and a clustering technique were used, in the program PCOrd (Mc Cune & Grace, 2002) to compare sites (individual trap yields) with respect to overall species composition based on the 15 most abundant ground beetles. DCA is a multivariate technique that positions samples along orthogonal axes that sequentially explain the greatest amount of inter-sample variation (Mc Cune & Grace, 2002). Default settings were used. All ground beetle species with more than 9 specimens or at least 6 individuals in at least one sampling station were used in the analyses, with transformed data (equal-weighting the species). A dendrogram was also constructed based on Euclidean distance (Ward's method, hierarchical grouping), as is recommended as a general-purpose linkage method that minimizes distortions in the underlying space. Very similar results were obtained with Sørensen distance and group average linkage and therefore only the first-mentioned dendrogram will be shown here. Interpretations were based on available knowledge on the biology, ecology and geographical distribution of the individual species (cf. Desender, 1989, and unpublished data; Turin, 2000).

Sampling for the population genetic studies (live-trapping) mainly took place during 2002 in Neupont and Chanly. For each of these two forests, 40 individuals were studied for *Abax ater* and *Carabus problematicus*. We already investigated genetic variability and differentiation with allozymes in both species from other forests in Belgium, with an emphasis on Flanders (Desender *et al.*, 1999, 2004, 2005b). Cellulose acetate electrophoresis was applied to study variability at enzyme loci (Hebert & Beaton, 1989). For more details on field sampling, electrophoresis and standard software for population genetic analyses, we refer to Desender & Verdyck (2001) and Desender *et al.* (1998, 2004).

Fig. 2 shows the detailed location of the three studied forest stands, against the background of historical maps of y1775 (de Ferraris), y1881 and y1923 (Institute for Military Cartography) and y1998 (National Geographic Institute). We also studied the map of y1854 (Van Der Maelen) but no major differences were observed with the map of y1881. Copies of most maps are in the collection of the RBINSc and several of these can be accessed also on the internet (http://patrimoine.met.wallonie.be/cartoth%C3%A8que/).

The situation at the time of de Ferraris (y1775) shows the Neupont site in continuous and mature deciduous forest (a situation persisting till today), while the Wellin site possibly made part of more or less shrub land. The Chanly site occurred in open agricultural land, possibly close to some remnants of (valley?) forest. By y1854 and y1881, only the Neupont site was forested, the Wellin site now being far from any persisting forest and the Chanly site close to some more or less larger parts of deciduous forest that gradually made contact to the large ancient forest to the South. At least from y1923, the Chanly site was enclosed in this large reforestation of mixed type, while the Wellin site was situated at the margins of (spruce?) forest and open land. Nowadays, all three forest stands are part of a more or less continuously forested large area, but only Neupont can be identified as ancient forest, whereas Chanly takes an intermediate position and Wellin seems most recent and closer to open habitat, also today.

Results and discussion

Ground beetle diversity, assemblages and forest history

Table 2 summarizes, per sampled forest, the obtained numbers of all ground beetles collected in pitfall traps. Fig. 3 presents the DCA ordination diagram for the 9 individual pitfall trap samples and for the 15 most abundant carabid species used for analysis, with ellipses enclosing samples from the same forest stand. Fig. 4 shows the obtained dendrogram based on the same data.

Overall species richness (s), somewhat surprising at first, is higher in the spruce plantation (s= 21) as compared to the mixed pine (s= 14) and deciduous forest (s= 15). However, inspection of the spruce stand species list shows this is at least partly due to the presence, in low numbers, of several non-forest ground beetles (e.g. *Pterostichus quadrifoveolatus*, a typical species for burned sites in coniferous forests, *Pterostichus diligens*, a species typical for fenland, *Pterostichus versicolor*, a species of acid grasslands). The higher diversity is i.o.w. possibly a consequence of edge effects (nearby open habitats; cf. Desender, 2005) as well as due to the presence of more or less open spots in this forest stand (cf. the presence of e.g. *Bembidion lampros, Carabus arvensis, Notiophilus biguttatus*).

DCA ordination shows that the largest part of variation in the data resides along the first axis (eigenvalue= 0.608), whereas the second axis has a much lower explanatory power (eigenvalue= 0.202). The first axis mainly opposes the (more recent and more disturbed) spruce stand at Wellin from the other forest plots. Part of these differences coincides not only with current forest ecological characteristics related to different tree species but also with a different age of continuous afforestation on the three sites and their immediate surroundings, ancient forest only being documented for the deciduous forest site and relatively close to the somewhat more recent mixed pine forest. The spruce forest site apparently made part of an area that has been deforested at several occasions during history and that only recently came into contact

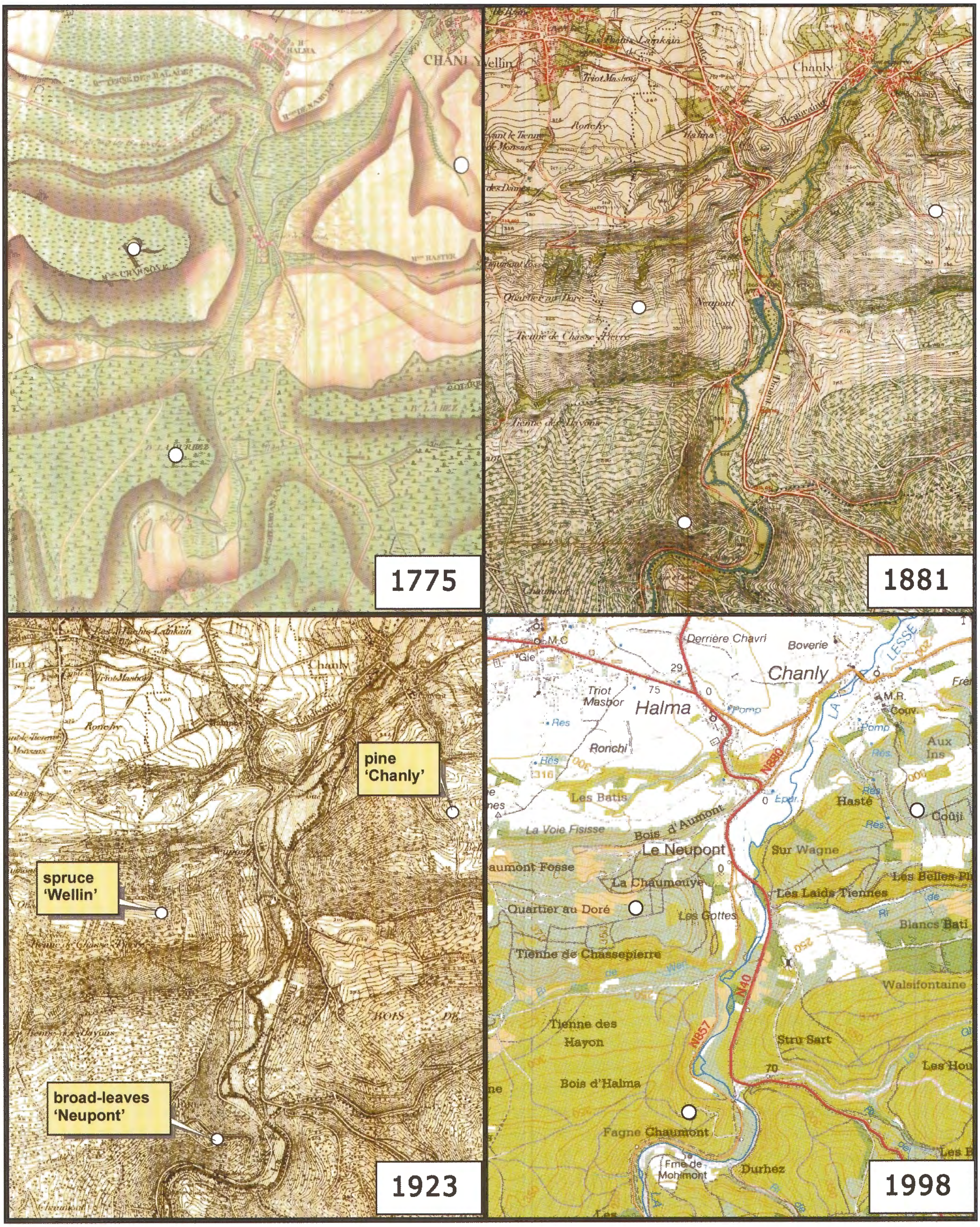

Fig. 2 — Historical maps from y1775 (de Ferraris; larger symbols in green areas symbolize mature forest (cf. Neupont site); smaller symbols probably to be interpreted as shrub land (cf. Wellin site)), y1881 and y1923 (Institute for Military Cartography) and y1998 (National Geographic Institute) with detailed localization of the three study sites (broad-leaves at Neupont, pine at Chanly and spruce at Wellin) (see text for further explanation).

Table 2 — Ground beetles obtained in the three study sites (sum of individuals in three pitfall traps) during 2002 (nomenclature after DESENDER et al., 1995)

site:	Neupont	Chanly	Wellin	
species:	**broad-leaves**	**pine-mixed**	**spruce**	**sum**
Abax ater (VILLERS, 1789)	41	137	6	184
Abax ovalis (DUFTSCHMID, 1812)	6	4	4	14
Abax parallelus (DUFTSCHMID, 1812)		62		62
Bembidion lampros (HERBST, 1784)		1	9	10
Carabus arvensis HERBST, 1784			1	1
Carabus coriaceus LINNAEUS, 1758	4	4	3	11
Carabus nemoralis O.F. MÜLLER, 1764	1	16	6	23
Carabus problematicus HERBST, 1786	61	20	34	115
Carabus violaceus purpurascens LINNAEUS, 1758			22	22
Cychrus attenuatus FABRICIUS, 1792	13	1	2	15
Harpalus quadripunctatus (DEJEAN, 1828)		1		
Harpalus rufipes (DE GEER, 1774)		2		1
Leistus rufomarginatus (DUFTSCHMID, 1812)			2	2
Loricera pilicornis (FABRICIUS, 1775)	1		2	2
Molops piceus (PANZER, 1793)		1		
Nebria brevicollis (FABRICIUS, 1792)	1	1	23	24
Notiophilus biguttatus (FABRICIUS, 1779)			8	8
Pterostichus cristatus (DUFOUR, 1820)	24			24
Pterostichus cupreus (LINNAEUS, 1758)	1	1		2
Pterostichus diligens (STURM, 1824)			1	1
Pterostichus madidus (FABRICIUS, 1775)	66	9	5	80
Pterostichus niger (SCHALLER, 1783)	11		32	43
Pterostichus oblongopunctatus (FABRICIUS, 1787)	20	96	19	135
Pterostichus quadrifoveolatus (LETZNER, 1852)			1	1
Pterostichus strenuus (PANZER, 1797)			1	1
Pterostichus versicolor (STURM, 1824)	1		1	2
Trichotichnus laevicollis (GYLLENHAL, 1827)			2	2
total individuals	**250**	**351**	**184**	**785**
number of species (s)	**14**	**15**	**21**	**25**

with older forest. The second DCA axis seems mainly related to the variability between the individual traps at Wellin. This corresponds to the, on the ground surface, somewhat more open detailed character of sample WEL3, with high numbers of *Bembidion lampros*. The dendrogram (Fig. 4) confirms the obtained assemblages as derived from the DCA and also clearly separates the different sampling units within their respective sampling stations. The cluster analysis further confirms that the spruce plantation has a more distant ground beetle assemblage as opposed to the two other study sites, whereas the variability between individual sampling units is also higher at this site, mainly due to the more distant position of sample WEL3 (Fig. 4).

Along the first DCA ordination axis, the three spruce sampling units are grouped at the right, with a corresponding higher number of the very common and eurytopic *Nebria brevicollis*, and the generalist forest species *Pterostichus niger.* At this site only, *Carabus violaceus purpurascens* was observed, a forest species that seems to be absent from more acid mineral-poor woodland. At higher elevation, this species has also been reported outside forest, accounting for the possibility to (re)colonize more recent forest in such regions (THIELE, 1977; TURIN, 2000; cf. DESENDER *et al.*, 2004). Other typical forest species occurred in several or all sites, but with differing numbers: *Abax ater, Carabus coriaceus, Carabus nemoralis* and *Pterostichus oblongopunctatus* (most in the intermediately-aged pine forest and in the ancient broad-leaves forest), *Abax ovalis*, *Carabus problematicus, Cychrus attenuatus* and *Pterostichus cristatus* (most or even exclusively in the ancient forest site Neupont), and *Abax parallelus* (exclusively at Chanly). Many of the last-mentioned species are known as forest interior species and in several other regions classified as ancient forest carabids (cf. ASSMANN, 1999; DESENDER *et al.*, 1999, 2005a). They all are constantly wingless (DESENDER, 1986a, 1989). Apparently, several of these

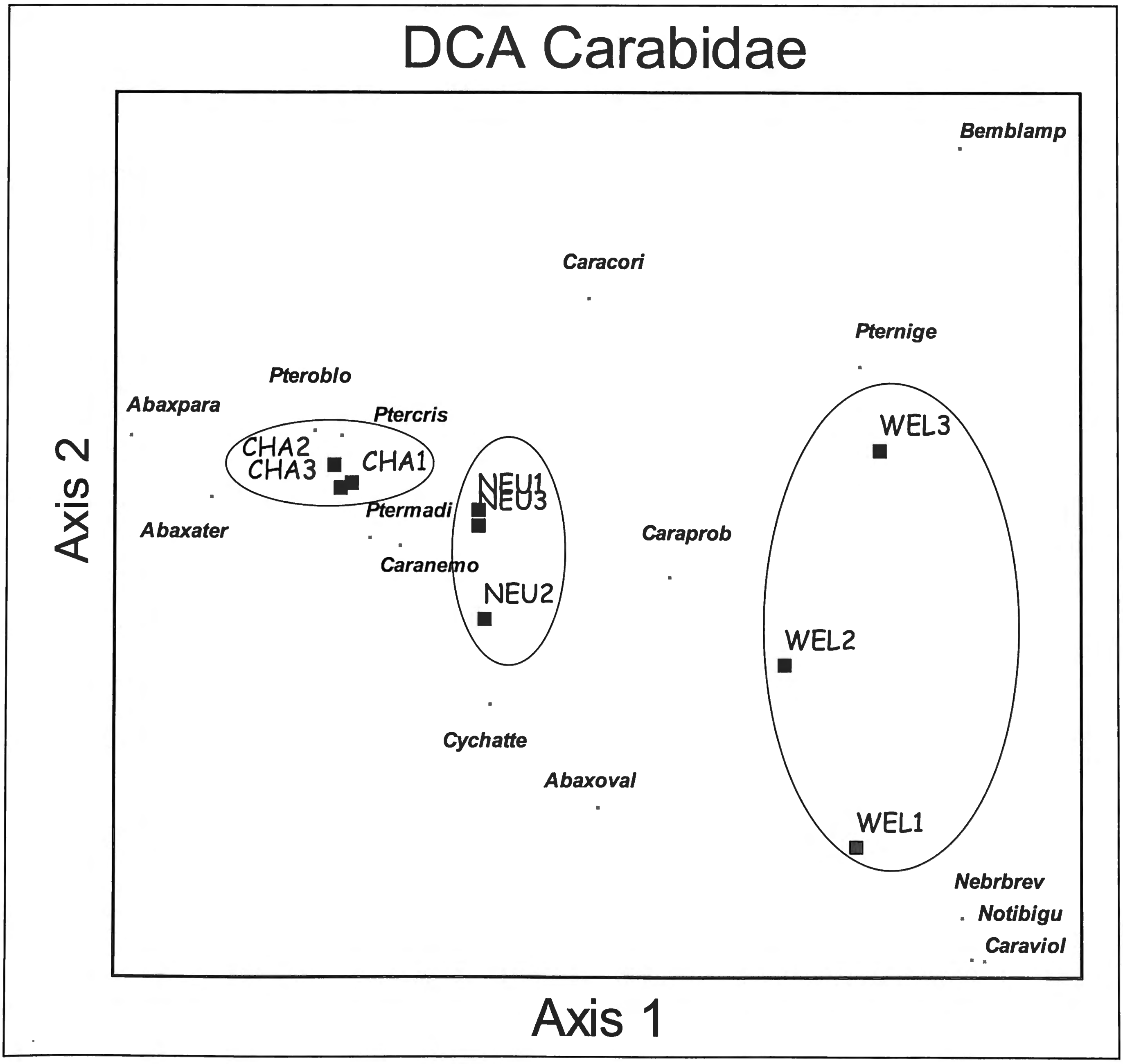

Fig. 3 — DCA-ordination species and sample scores based on the 15 most abundant carabid species obtained in the individual traps of the three sampled forest sites (species name abbreviations show first 4 letters of genus name and first 4 of species name; site abbreviations CHA (Chanly, mixed pine forest), NEU (Neupont, broad-leaves) and WEL (Wellin, spruce) are followed by trap number; added ellipses enclose individual sampling units per site.

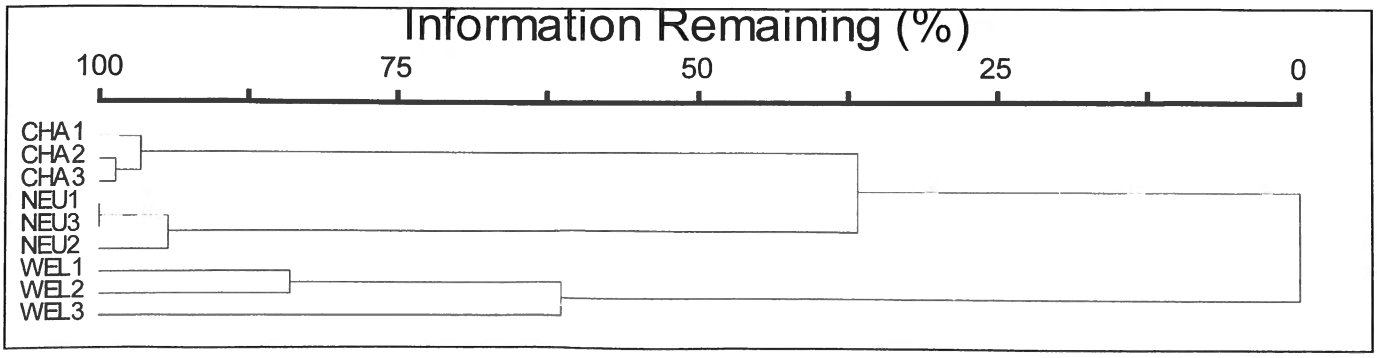

Fig. 4 — Dendrogram of the samples based on Euclidean distance between species assemblages (Ward's method of hierarchical grouping).

ground beetles have managed to (re)colonize the more recent forest plots in this study area, although several species still show a pronounced preference for the ancient plot. This is not surprising in view of the recent reforestation in this region (see higher), because forest expansion took place in close proximity or immediately adjacent to a large ancient forest area. Although effects of silvicultural practices have much impact on forest ground beetle assemblages, as recently shown in some large scale studies on Wallonian forests (cf. DE WARNAFFE & LEBRUN, 2004; PONTÉGNIE *et al.*, 2005), past land use has also impact on forest soils and biodiversity, sometimes even in a long-term irreversible manner (DUPOUEY *et al.*, 2002; DUPOUEY & DAMBRINE, 2006).

Ground beetle dispersal power in the three study sites.

Fig. 5 summarizes the dispersal power of ground beetles in each of the sampling sites (brachyptery versus macroptery, with separate indication of wing polymorphic species) respectively on species and on individual level. *Trichotichnus laevicollis* is one of the very few carabids in Belgium displaying sex-linked wing dimorphism: males always have wings, whereas females are invariably wingless (cf. DESENDER, 1987).

As expected, wingless ground beetles dominate in the ancient forest plot, this trend being more strongly visible on the graph based on relative number of individuals (cf. DESENDER, 1989). On the other hand, we can clearly observe that the influenced spruce forest shows a much higher amount of species and individuals belonging to wing polymorphic and constantly macropterous carabids. Previous studies in Belgium have shown that in the category of wing polymorphic ground beetles there is a significantly higher amount of eurytopic species with lower nature conservation value (DESENDER, 1986b) and these data thus confirm the lower species quality of spruce plantations.

Species found in very low numbers on a given site will probably indicate accidentally immigrating individuals from surrounding habitats (DESENDER, 1996). The single macropterous *Pterostichus diligens* (a species normally mainly found with short-winged individuals) is a clear example. Sustainable conservation and forest management therefore preferably should focus on species quality and population size instead of concentrating only on total species richness. Other aspects of target species, such as population genetic diversity and/or dispersal power, are further recommended for valuable additional information in evaluation and monitoring studies.

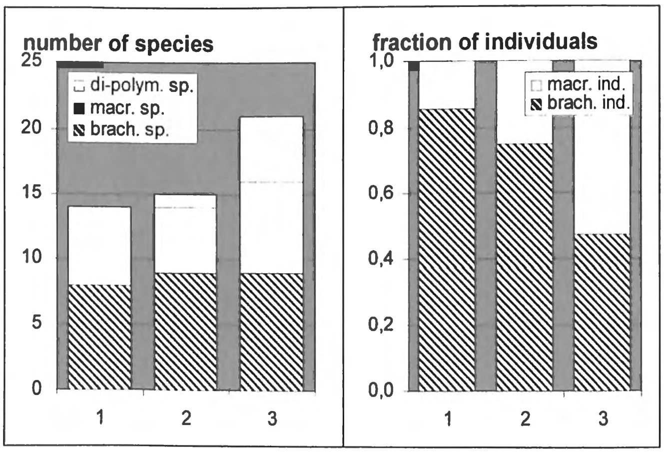

Fig. 5 — Ground beetle dispersal power on species level (left graph) regrouped per sampled forest (brach.= brachypterous, di-polym.= wing dimorphic or polymorphic, macr.=macropterous or full-winged); right graph shows brachypterous and macropterous proportion of ground beetle individuals. Sites: 1= ancient deciduous forest at Neupont, 2= mixed pine forest at Chanly, 3= spruce plantation at Wellin.

Genetic diversity and differentiation in ***Abax ater*** *and* ***Carabus problematicus.***

The population genetic data showed no deviations from Hardy-Weinberg equilibrium and no linkage disequilibrium, which means that the studied enzymes can be used as independent markers.

Gene diversity (H_{exp}) of *Abax ater* was 0.157 at Chanly and 0.173 at Neupont. Locus-specific genetic differentiation tests and inspection of allelic frequencies showed a significant difference between the two sites for one locus (MPI; p=0.0071), with more equilibrated alleles at Neupont based on the three (same) alleles observed in both populations (Fig. 6). Overall genetic differentiation between both sites was also statistically significant (F-st(theta)= 0.031; p<0.01).

Gene diversity (H_{exp}) of *Carabus problematicus* was 0.188 at Chanly and as high as 0.331 at Neupont, this important difference being visible simultaneously in the genetic differentiation based on three independent loci (G6PDH, p= 0.0021; IDH2, p= 0.0047; PEP-Z, p= 0.0004). Allelic differences are illustrated for these enzymes in Fig. 6 and show, on top of clearly differing frequencies, the presence of unique alleles for all three allozymes in beetles from Neupont as compared to Chanly. Overall genetic differentiation was also significant between both samples (F-st(theta)= 0.024; p<0.01).

These parallel population genetic studies on the forest carabids *Abax ater* and *Carabus problematicus* showed, for both species, a high genetic diversity in the ancient deciduous forest plot, as compared to results from other forests in Belgium (DESENDER *et al.*, 2004, 2005b). This is probably a reflection of the relatively large and continuous forest surfaces, which have been present in this area for a very long time. Furthermore, a low genetic diversity is, for both species, observed in the more recent mixed forest site, probably as a result of a relatively recent colonization history from a restricted number of individuals. Whether these differences are purely neutral and/or adaptive will be investigated in the future (microsatellites, morphometrics).

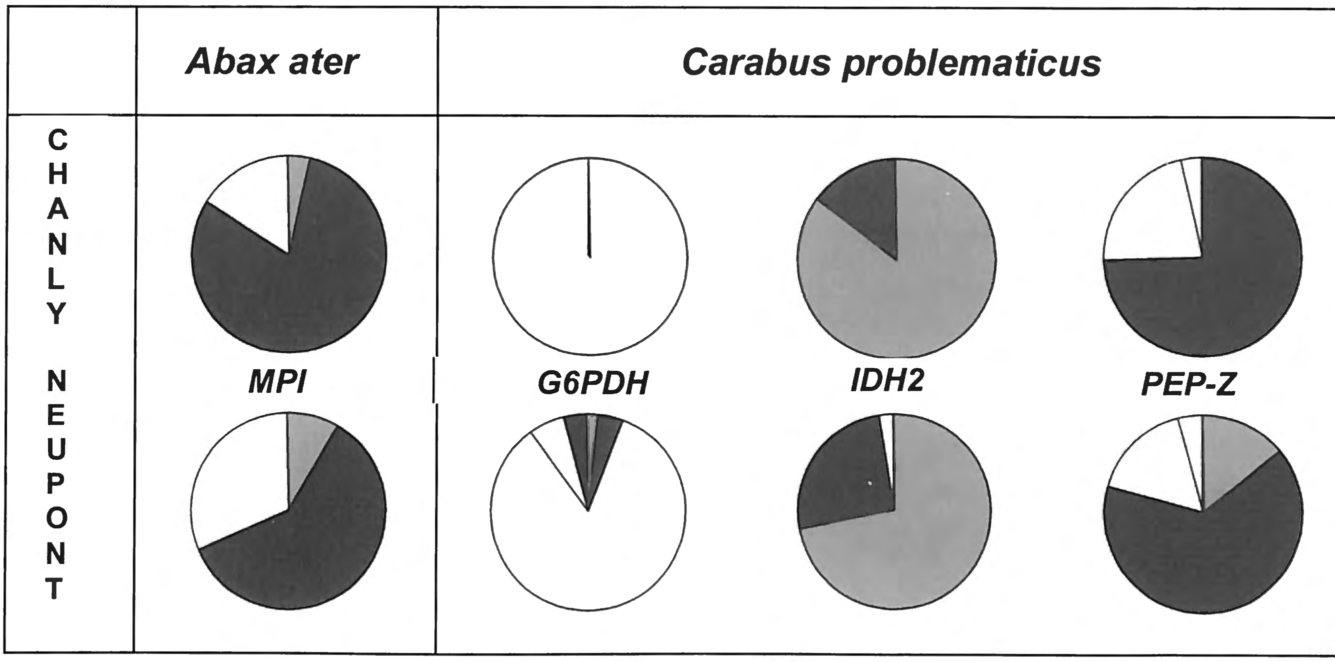

Fig. 6 — Observed allele frequencies for *Abax ater* and *Carabus problematicus* compared for 4 allozymes between the ancient forest (Neupont) and the more recent mixed pine forest (Chanly); allele frequencies derived from n=80 in each case (see text for further explanation).

Conclusions

Ground beetle assemblages mainly differentiated between the more recent spruce forest at Wellin and the other two sites, which were dominated by more specialized forest species. At Wellin, a site heavily perturbed by wild boar, and close to open habitats, several more general ground beetle species were encountered, indicative for disturbed and/or more open forest sites. We observed that land use during history is not independent of current forest type on these respective plots. This phenomenon is supposed to be of great importance in the current distribution of typical forest beetle species (cf. DESENDER *et al.*, 1999, 2004). Both sets of factors cannot easily be studied independently and will be evaluated in the future on a much larger dataset from different eco-regions in our country (DESENDER *et al.*, in prep.). We conclude that, in the studied area, typical forest ground beetle assemblages have been able to establish themselves, sometimes in low numbers, on sites, which were not forested about 250 years ago. Our genetic results, however, warn that such recent forest populations can be genetically impoverished. Forest history as well as ecology and management therefore seem to have an impact not only on observed ground beetle species, but also on the intraspecific genetic diversity levels.

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