



Ecological differentiation and reproductive isolation of two closely related sympatric species of *Oenanthe* (Apiaceae)

ERIK WESTBERG¹*, HANS-HELMUT POPPENDIECK² and JOACHIM W. KADEREIT¹

¹Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg-Universität Mainz, D-55099 Mainz, Germany

²Biozentrum Klein Flottbek und Botanischer Garten, Ohnhorststrasse 18, Universität Hamburg, D-22609 Hamburg, Germany

Received 28 December 2009; revised 19 April 2010; accepted for publication 20 April 2010

Oenanthe conioidea is a lower Elbe endemic plant species growing in the freshwater tidal zone around Hamburg (Germany). Its closest relative *Oenanthe aquatica* is widely distributed in Eurasia and grows in calm and shallow freshwater. The two species differ in habitat requirements but are otherwise sympatrically distributed, suggesting that ecological divergence has to be maintained in the face of gene flow. In the present study, we investigated ecological differentiation and reproductive isolation in these two species. An amplified fragment length polymorphism analysis found clear genetic differentiation between the two species implying reproductive isolation. A reciprocal transplantation experiment including artificial F_1 hybrids showed strong selection against immigrants. In the two parental habitats, the non-native species are less fit than the native species. Hybrids are less fit in the habitat of *Oe. aquatica* but perform as well as the native species in the habitat of *Oe. conioidea*. We hypothesize that selection against immigrants is the most important component of reproductive isolation between the two species, and that selection against immigrants is the result of exposure to cold and wind in the tidal habitat of *Oe. conioidea* and to herbivory in the habitat of *Oe. aquatica*. These results are congruent with a role for differential ecological selection in the formation and maintenance of these two species. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, 101, 526–535.

ADDITIONAL KEYWORDS: AFLP – divergence – local adaptation – transplantation experiment – water dropworth.

INTRODUCTION

Reproductive isolation is required for ecological divergence because of the homogenizing effect of gene flow (Schluter, 2000). Early stages of ecological differentiation are often found to be accompanied by pre-mating reproductive barriers in form of habitat or temporal isolation, whereas intrinsic postzygotic barriers are considered to take longer to evolve, especially among sympatric taxa (Coyne & Orr, 1997, 2004). In addition to inviability or reduced fertility of first- or later-generation hybrids, postzygotic barriers can also exist in form of ecological inviability when hybrids show reduced fitness in the habitat of their

parental taxa (Schluter, 2000). Habitat isolation and ecological hybrid inviability can thus arise when populations of a species adapt to a new, spatially separate habitat. Sister species of young age usually are allopatrically distributed (Barraclough & Vogler, 2000; Bolnik & Fitzpatrick, 2007), and this is even more often the case for intraspecific taxa that often are recognized partly based on their allopatric distributions (Coyne & Orr, 2004). Occasionally, closely related taxa occur in sympatry, and such cases represent interesting opportunities to investigate how species can maintain their integrity when living in close vicinity.

Oenanthe aquatica (L.) Poiret is a widespread plant species growing in standing or slow-flowing freshwater habitats. Its close relative *Oenanthe conioidea* (Nolte

*Corresponding author. E-mail: westberg@uni-mainz.de

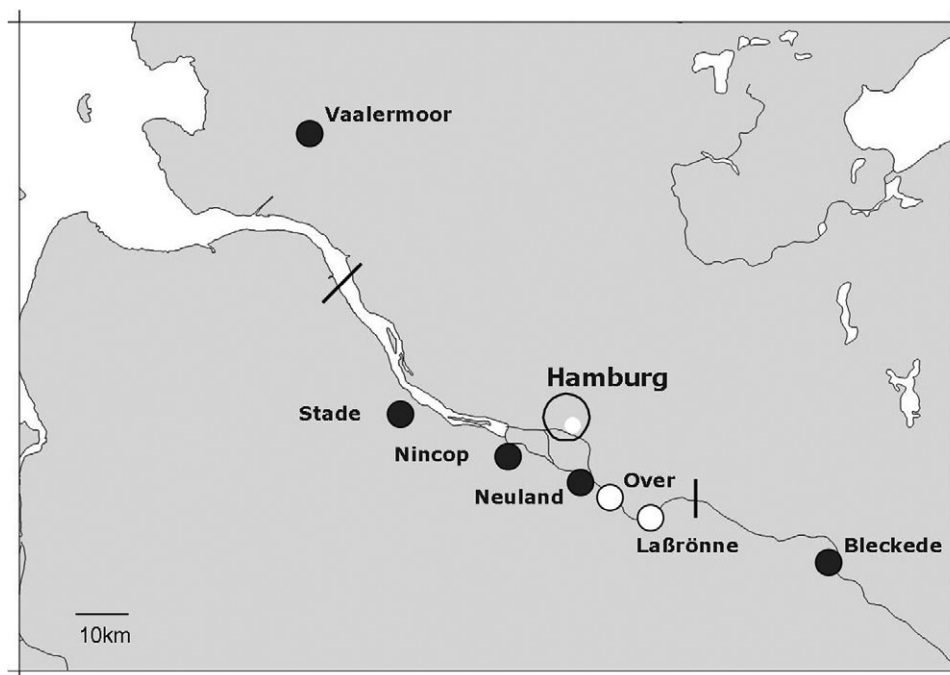


Figure 1. Geographical location of populations where samples for amplified fragment length polymorphism analysis were collected. Dark circles represent *Oenanthe aquatica* populations and white circles represent *Oenanthe conioides* populations. The lines delimit the potential distribution range of *Oe. conioides* (freshwater tidal habitats).

ex Rchb.f.) Lange is a narrow endemic of the lower Elbe River and is found in habitats characterized by fresh water tidal movements (Kadereit & Kadereit, 2005). This unusual habitat property is caused by the discharge of the river being blocked by the high tide of the North Sea and the subsequent outflow at low tide. *Oenanthe conioides* is severely threatened (Ludwig & Schnittler, 1996; Walter & Gillet, 1998), primarily by dyke construction resulting in habitat loss and changes in the tidal regime (Below, Poppendieck & Hobohm, 1996; Below & Hobohm, 1998; Below, 1999).

The two biennial taxa can be distinguished mainly by leaf shape and ecology. *Oenanthe aquatica* has more finely dissected leaves than *Oe. conioides*, and underwater leaves are usually present in *Oe. aquatica* but not in *Oe. conioides*. The morphological difference has been interpreted as adaptive (Junge, 1912). Whereas the habitat of *Oe. aquatica*, irrespective of seasonal variation of water level, can be regarded as comparatively stable and sheltered, the freshwater tidal habitat of *Oe. conioides* is subject to substantial diurnal and seasonal disturbance. Diurnal disturbance includes the flooding and exposure of plants twice a day and seasonal disturbance includes much stronger exposure (compared to the *Oe. aquatica* habitat) to low temperatures and strong winds. On the basis of an analysis of the phylogenetic relationships between *Oe. conioides* and *Oe. aquatica*, Kadereit & Kadereit (2005) concluded that *Oe. conioides*

is an evolutionary lineage that is nested within *Oe. aquatica*. On the basis of the absence of internal transcribed spacer (ITS) variation between the two species, Kadereit & Kadereit (2005) interpreted *Oe. conioides* as a geologically very young derivative of *Oe. aquatica*. Furthermore, the narrow distribution area of *Oe. conioides* is completely enclosed by that of *Oe. aquatica*, and populations of the two species often grow close to each other (Fig. 1).

This setting implies that the two species diverged ecologically through the colonization of a different habitat by *Oe. conioides*, and that this divergence has to be maintained in the face of gene flow. The present study investigated the adaptive divergence and genetic differentiation of these two species. In a reciprocal transplantation experiment, we investigated whether the two species are adapted to their respective habitats. We included experimentally produced interspecific F_1 hybrid individuals in the transplantation experiment to analyze their performance in the two parental habitats. The transplant experiments were not only carried out aiming to detect overall adaptive differentiation, but also to obtain additional information on the influence of selection on reproductive isolation and additional information on the relevant selective forces in the two habitats. Furthermore, we investigated genetic differentiation between *Oe. conioides* and several populations of *Oe. aquatica* to determine the extent of reproductive isolation between the

Table 1. Origin of population samples for the amplified fragment length polymorphism analysis and genetic diversity estimates

Population	<i>N</i>	Latitude	Longitude	<i>P</i>	<i>H_E</i>
<i>Oenanthe coniooides</i>					
Over	15	53°25'	10°06'	79.0%	0.251
Laßbrönne	15	53°24'	10°14'	71.8%	0.228
<i>Oenanthe aquatica</i>					
Bleckede	17	53°19'	10°53'	70.2%	0.244
Nincop	21	53°30'	10°48'	68.6%	0.250
Neuland	16	53°27'	10°02'	77.4%	0.270
Stade	18	53°36'	9°31'	73.4%	0.257
Vaalermoor	16	54°00'	9°20'	71.0%	0.252

P, percent polymorphic loci; *H_E*, expected heterozygosity.

two species using amplified fragment length polymorphism (AFLP) analysis.

MATERIAL AND METHODS

PLANT MATERIAL

Leaf material for molecular analyses was collected in the field in autumn and winter 2006 and dried on silica gel. Between 15 and 21 individuals were sampled from five populations of *Oe. aquatica* and two of *Oe. coniooides* (Fig. 1, Table 1). The populations of *Oe. aquatica* from Neuland, Nincoper Moor and Stade are located near the distribution area of *Oe. coniooides* and henceforth are referred to as sympatric. The populations from Vaalermoor and Bleckede are referred to as allopatric. For the transplantation experiment, seeds of *Oe. aquatica* were collected from a population in the Neuland area, approximately 1 km from the transplantation site, and seeds of *Oe. coniooides* were collected from Over, both in 2004. *F₁* hybrids were obtained from hand crosses between plants that were collected in the field and transported to the Botanical Garden of Johannes Gutenberg-Universität Mainz, where the crosses were made. Pollen recipients were emasculated before crossing, and the resulting seeds were collected and stored until the start of the experiments. Seeds were not sown in the transplant sites because of the risk of displacement or removal by tides and water movement. In March 2005, seeds were germinated in the greenhouse and transferred into pots and moved outdoors until planting them in the field in May. Fifty individuals each of *Oe. aquatica*, *Oe. coniooides* and their *F₁* hybrid were planted into each habitat. The original aim was to plant five siblings from ten families (a family is defined here as the progeny of one mother individual) per species and their hybrid. Because of uneven germination success, however, the number and size of families varied. In the end, nine families of *Oe. aquatica* of four to seven

individuals, seven families of *Oe. coniooides* of five to 12 individuals, and five *F₁* families of ten full-sibs each were planted into each habitat. All families were used at both sites.

AFLP ANALYSIS

The AFLP protocol was performed *sensu* Kropf, Kadereit & Comes (2003), who used a modification of the protocol of Vos *et al.*, (1995). Approximately 150 ng of DNA was simultaneously restricted and ligated to adaptors (*Eco*RI, 5'-CTCGTAGACTGCGTACC-3'/5'-AATTGGTACGCAGTC-3'; *Mse*I, 5'-GACGATGAGTCCTGAG-3'/5'-TACTCAGGACTCT-3') at 23 °C for 14 h. Pre-selective amplification was performed using primers E01 (5'-GACTGCGTACCAATTCA-3') and M02 (5'-GATGAGTCCTGAGTAAC-3'). For the selective amplifications, the primer combinations E37/M57 (E01 + CG/M02 + GG), E39/M49 (E01 + GA/M02 + AG) and E45/M57 (E01 + TG) were used. The AFLP products were separated on 6% polyacrylamide gels as a multiplex of three primer combinations labelled with fluorescent dyes (6-FAM, NED, and HEX; Applied Biosystems), together with an internal size standard labelled with ROX (ROX 500; ABI). Gels were run for approximately 4 h on an ABI 377 automated sequencer. Gels were scored automatically with GENEMAPPER, version 3.5 (Applied Biosystems), and corrected manually. Ambiguities were recorded as missing data. Genotyping error rates were estimated with eight replicated samples by dividing the number of mismatches between replicates by the total number of phenotypic comparisons (Bonin *et al.*, 2004).

Statistical analysis

Genetic diversity was measured as number of polymorphic loci, *P*, and expected heterozygosity, *H_E*, and was estimated with GenAlEx, version 6 (Peakall & Smouse, 2006).

Spatial genetic structure was analyzed with a Bayesian, individual-based clustering method implemented in BAPS, version 4.1 (Corander, Waldmann & Sillanpää, 2003; Corander, Marttinen & Mäntyniemi, 2006). This method clusters individuals into groups assuming Hardy–Weinberg equilibrium and linkage equilibrium within clusters. The number of clusters and their allele frequencies are estimated jointly. The analysis was run ten times each for $K=2$ to 10, where K is the assumed maximum number of clusters in the data. To quantify population and taxon differentiation, a hierarchical analysis of molecular variance (Excoffier, Smouse & Quattro, 1992) based on marker frequencies was performed using GenAlEx, version 6. Genetic variation was partitioned among taxa, among populations within taxa and among individuals.

Mantel tests (Mantel, 1967) for correlation between geographical and genetic distances (isolation-by-distance) were performed using GenAlEx, version 6, with 9999 permutations. If populations of the two species are connected by gene flow and dispersal is limited, one would expect to find an isolation-by-distance pattern both when all populations are analyzed together and when excluding populations of *Oe. coniooides*. Genetic distance was measured as $\Phi_{st}/(1 - \Phi_{st})$ and geographical distance was \ln transformed *sensu* Rousset (1997).

TRANSPLANTATION EXPERIMENT

Plants were randomly transplanted into two sites corresponding to *Oe. coniooides* and *Oe. aquatica* habitats. Because of *Oe. coniooides*' status as a globally threatened plant, we could not perform the experiment in a site of a natural population of *Oe. coniooides*. For similar reasons, we were unable to replicate the experiment at additional sites. The *Oe. coniooides* habitat used (Haken) is an old port basin in central Hamburg that has been partially filled and renaturalized. Although *Oe. coniooides* does not naturally occur there, the vegetation at this site is typical of fresh water tidal habitats in the Elbe and was considered suitable for our experiment based on both hydrological and vegetational considerations. Here, our experimental plants were placed in chicken wire cages to minimize the chance of individuals being flushed away by storm tides, which possibly could lead to genetic contamination of natural populations. The *Oe. aquatica* habitat was a wide ditch with shallow water between pastures, approximately 1 km away from the river Elbe in the Neuland area. This habitat harboured a few naturally occurring individuals of *Oe. aquatica* in 2005 but not in 2006. Plants were transplanted along a transect.

Data were collected approximately monthly in the summer and autumn and less often in winter and spring. No data were collected in Neuland in February 2006 because the site was covered by ice. At each visit, (1) survival, (2) number of rosette leaves, (3) length and (4) width of the largest leaf were recorded and, in flowering individuals, the (5) number of flowers per plant was estimated. For determining the number of flowers, the number of flowers on three umbellets randomly chosen from each branching order of umbels, the number of umbellets on three random umbels per branching order, and the total number of umbels for each branching order were counted. These figures were used to calculate the number of flowers for each branching order, and the results for the different branching orders were added. The estimate was square-root transformed before further analysis. To avoid accidental gene flow between experimental and natural populations of *Oe. coniooides*, plants were harvested after the three first branching order umbels had started to form flowers large enough to be counted in the *Oe. coniooides* habitat.

Statistical analysis

The mean number of flowers produced by surviving individuals was estimated in each habitat by fitting the data to a model with restricted maximum likelihood with taxon/ F_1 hybrid as fixed effect and individuals as random effect using GenStat, version 10.1 (VSN International Ltd). In this analysis, relatedness between individuals was accounted for by a covariance matrix where F_1 families were assumed to be full-sibs and field-collected seeds from a single mother were considered as half-sibs. Fitness was analyzed with the software ASTER (Geyer, Wagenius & Shaw, 2007). ASTER implements a maximum likelihood method to combine and analyze multiple fitness components. The ASTER models account for dependencies between fitness components and can incorporate variables that have different probability distributions. This method was used to combine the analysis of two different fitness components: survival to flowering and the number of flowers. For each habitat, an analysis was conducted using ASTER to compare the performance of the native and alien taxa. Two dependent fitness components (i.e. survival to flowering and number of flowers) were combined in the analysis. The models included spatial position in the field site and taxon as independent variables. To test for differences between the parental taxa in fitness, we compared a model including the effect of taxon on the total number of flowers to a model without this effect with a likelihood ratio test. In this analysis, survival is of interest only insofar as it has an effect on the number of flowers and this is

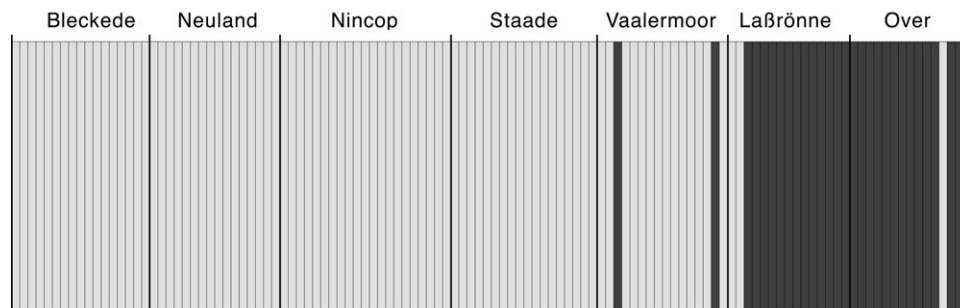


Figure 2. Results of the Bayesian clustering analysis of *Oenanthe coniooides* (dark) and *Oenanthe aquatica* (light). Each individual is represented by a vertical bar, and bars of the same shading belong to the same cluster.

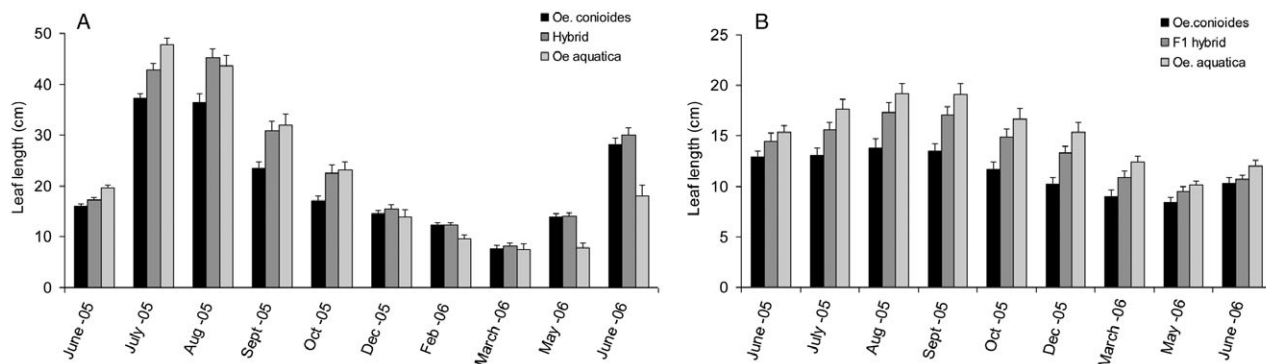


Figure 3. Length of the largest leaf from the *Oenanthe coniooides* (A) and *Oenanthe aquatica* (B) habitats. Error bars indicate one standard error.

accounted for by the ASTER model. A separate comparison was carried out for the F_1 hybrid and native taxon at each site. The models for this analysis contained the same variables as described for the taxon comparisons. Mean values for total number of flowers and their 95% confidence intervals for the two species and their hybrid were predicted for a hypothetical individual having a central spatial position in the field sites and by analysing species and hybrids together.

RESULTS

GENETIC DIVERSITY

A total of 124 loci were scored for 118 individuals. The error rate based on eight replicated samples was 0.01. Eighty-nine percent of the loci were polymorphic at the 99% level. Genetic diversity was similar among all populations examined (Table 1), with an average expected heterozygosity of 0.24 and 0.25 for *Oe. coniooides* and *Oe. aquatica*, respectively.

The Bayesian cluster analysis identified two clusters which corresponded to the two species and showed that the individuals of the two species are mostly distinct from each other (Fig. 2). Two individu-

als from an *Oe. aquatica* population and three from *Oe. coniooides* populations were misclassified in the BAPS analysis. A hierarchical analysis of molecular variance (AMOVA) partitioned 5.0% ($P < 0.001$) of the variation among taxa and 5.2% ($P < 0.001$) among populations within species. The correlation between geographical and genetic distances did not indicate an isolation-by-distance pattern among populations. When including all populations, the correlation was $r = -0.15$ ($P = 0.33$) and, when *Oe. coniooides* populations were excluded, the correlation rose only slightly to $r = 0.25$ ($P = 0.27$).

TRANSPLANTATION EXPERIMENT

The growth parameters measured (length and width of the largest rosette leaf, number of rosette leaves) showed similar trends during the course of the experiments and were partly correlated with each other (see Supporting information, Figs S1, S2, S3, S4). Subsequently, leaf length is used to represent growth parameters (Fig. 3). At both sites, there was an increase in growth during the summer followed by a decline in autumn and winter. In spring, plants generally showed an increase in growth, although the

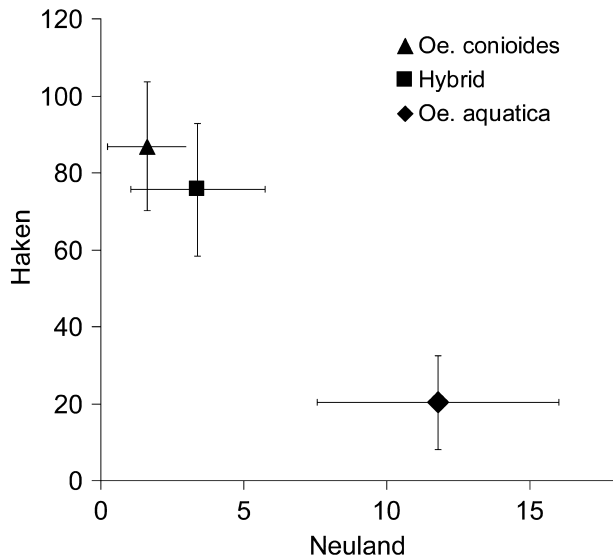


Figure 4. Estimated mean of the square root-transformed number of flowers of the three taxa at the two experimental sites. Bars indicate 95% confidence intervals.

recovery after the winter was less pronounced in *Oe. aquatica* at the *Oe. coniooides* site. Although this pattern was found in both habitats, the changes in growth parameters were larger in the *Oe. coniooides* site. The decline in leaf size recorded in September and October in the *Oe. coniooides* habitat was caused by grazing by waterfowl (E. Westberg, pers. observ.). Growth parameters in the *Oe. aquatica* habitat were also influenced by grazing, mainly by snails, although this took place throughout the year with the exception of the coldest months (E. Westberg, pers. observ.).

At the *Oe. aquatica* site, 25 individuals at one end of the transect died before the first visit and were excluded from the fitness analyses. The predicted mean number of flowers of the ASTER analyses showed that the native taxon is fitter than the alien species in its respective habitat (Fig. 4). The differences found in the total number of flowers between taxa were significant in both habitats (Neuland: deviance = 25.0, d.f. = 1, $P < 0.001$; Haken: deviance = 37.0, d.f. = 1, $P < 0.001$). The hybrids showed intermediate fitness compared to their parental species in both habitats (Fig. 4). In the *Oe. aquatica* habitat, hybrids produced significantly fewer flowers than the native species (deviance = 13.9, d.f. = 1, $P < 0.001$) but this difference was smaller and not significant at the *Oe. coniooides* site (deviance = 1.0, d.f. = 1, $P = 0.35$). Comparisons between models with or without taxon identity of the maternal parent of the hybrids as a predictive variable did not provide evidence that this had an effect in the experiments (Neuland: devi-

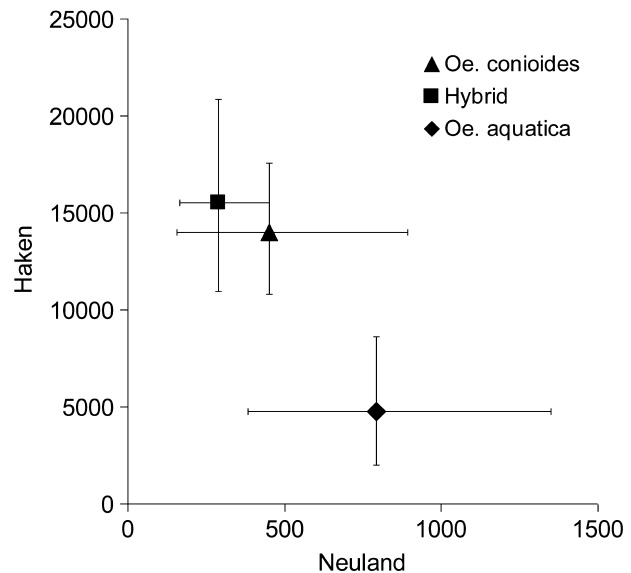


Figure 5. Mean number of flowers with 95% confidence intervals of surviving plants at the two experimental sites.

ance = 3.6, d.f. = 1, $P = 0.1$; Haken: deviance = 0.09, d.f. = 1, $P = 1$). When only surviving individuals were considered at the *Oe. coniooides* site, F_1 hybrids showed similar or slightly higher fitness than the native species, whereas, in the *Oe. aquatica* habitat, hybrids produced markedly fewer flowers than the native species (Fig. 5).

Overall, both survival and number of flowers was higher (Figs 5, 6) and vegetative traits were larger (Fig. 3) at the *Oe. coniooides* site. This is probably an effect of the higher productivity of the tidal habitat (Meyer, 1957) rather than of habitat related fitness of the plants themselves.

DISCUSSION

GENETIC STRUCTURE

The analysis of AFLP variation clearly showed that the two taxa are genetically distinct. In the individual-based Bayesian clustering analysis, samples of *Oe. coniooides* clustered together to the exclusion of samples of *Oe. aquatica*, and the AMOVA showed significant differentiation between populations of the two species. We found no correlation between geographic and genetic distances when using either all populations or only populations of *Oe. aquatica*. Thus, the populations investigated are not primarily structured by an isolation-by-distance mode of dispersal. This may imply that genetic drift has a large influence on spatial genetic patterns because populations of both species usually do not persist for long periods of time. *Oenanthe aquatica* will disappear when its habitat

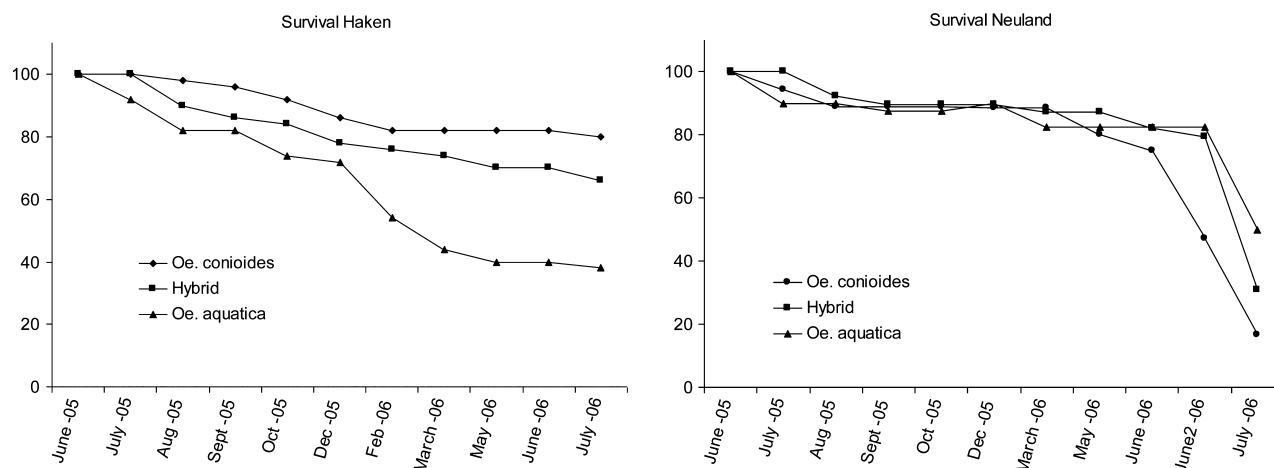


Figure 6. Percentage surviving individuals during the transplantation experiment. Numbers for the last month indicate flowering individuals.

becomes overgrown or water regimes change (Hroudová *et al.*, 1992), and *Oe. coniooides* is susceptible to both the fortuitousness of its habitat and to the vegetational succession along the river banks.

A few individuals of *Oe. coniooides* were misclassified in the Bayesian clustering analysis. This can be interpreted as evidence for interspecific gene flow. Although migration of individuals between habitats and their establishment is plausible in view of the close proximity of the two species and the results from our transplantation experiment, misclassification of individuals should be viewed with caution. Misclassification could instead indicate that the number of AFLP fragments used in the analysis was too low to successfully assign all individuals given the level of between species differentiation found here. The finding that two individuals of *Oe. aquatica* from Vaalermoor clustered with *Oe. coniooides*, even though the Vaalermoor site (Fig. 1) lies well outside the current distribution area of *Oe. coniooides* and 20 km away from the Elbe river, also supports the interpretation of misclassified individuals as a chance result rather than as the result of gene flow. The gene flow hypothesis, however, cannot be rejected based on our data.

The results obtained in the present study fully confirm those of an earlier study by Kadereit & Kadereit (2005), who also found that the two taxa fall into separate genetic clusters in an AFLP analysis of 30 individuals of *Oe. coniooides* and *Oe. aquatica*. Using *Oe. fluviatilis* as outgroup, the authors additionally found *Oe. coniooides* to be nested within *Oe. aquatica* in a Neighbour-joining dendrogram. On the basis of this finding, they interpreted *Oe. coniooides* as an evolutionary derivative of *Oe. aquatica* as the more widespread progenitor species.

Although *Oe. coniooides* and *Oe. aquatica* are genetically clearly distinct, the amount of variation partitioned among taxa is rather small and comparable to the amount of variation partitioned among populations within taxa as reported in other studies. Compared to other studies using dominant markers, both species show less differentiation than short-lived perennials and both endemic and widely distributed species (Nyblom, 2004). On the other hand, estimates of H_E in both species were similar to those of other studies (Nyblom, 2004).

LOCAL ADAPTATION AND MAINTENANCE OF SPECIES INTEGRITY

The reciprocal transplantation experiment showed that the two species are adapted to their respective habitats and that there is selection against both nonconspecific immigrants and F_1 hybrids in the *Oe. aquatica* habitat and at least against nonconspecific immigrants in the *Oe. coniooides* habitat. On the basis of our experiments, we hypothesize that the reproductive isolation between the two species as suggested by our AFLP data is the result of a combination of strong selection against immigrant individuals from the respective other habitat, and reduced ecological viability of hybrids, at least in the *Oe. aquatica* habitat. Selection against hybrids in the *Oe. coniooides* habitat may take place in later hybrid generations (see below), which were not included in our experiments. Because our crosses between *Oe. aquatica* and *Oe. coniooides* to produce F_1 hybrids were successful, and these hybrids produced normal seeds, there appear to be no obvious intrinsic postzygotic barriers to hybridization, at least in the F_1 generation.

When considering selection against the F_1 hybrids it should be noted that, although the total fitness of the hybrid population is slightly reduced in the tidal habitat, those hybrid individuals that survived showed mid-parent heterosis (i.e. their fitness is equal to the fittest parent, and the overall fittest individuals encountered were hybrids). Arnold (1997) noted that heterosis is commonly seen in hybrids and thus heterosis is also likely to be common during incipient speciation between locally adapted populations. It remains unclear, however, whether individual heterosis plays a large role in opposing local adaptation and differentiation between populations. The data obtained in the present study imply that, in our case, heterosis does not hinder local adaptation. Although heterosis on the one hand can increase effective gene flow (Ingvarsson & Whitlock, 2000), on the other hand, it breaks down rapidly after the first hybrid generation (Rieseberg, Baird & Gardner, 2000), and later-generation hybrids may show outbreeding depression that was masked by heterosis in the F_1 -generation (Edmands, 1999; Whitlock, Ingvarsson & Hatfield, 2000; Kawecki & Ebert, 2004).

THE NATURE OF DIVERGENT SELECTION

When considering the data from the present study, the two habitats appear to differ in more aspects than tidal inundation versus stable water level. When the vegetative performance of the plants (Fig. 3) and the timing of deaths (Fig. 6) in the two habitats are considered, several differences can be observed. In the *Oe. coniooides* habitat, the mortality of the alien species was highest during the coldest months, whereas mortality was more or less constant in the native species and the F_1 hybrids. Presumably, this was caused by exposure to cold and wind during low tide in the *Oe. coniooides* habitat, to which *Oe. aquatica*, protected by submersion in water in its native habitat, appears to be less tolerant. After the cold period ended, *Oe. aquatica* individuals did not regain biomass as quickly as *Oe. coniooides* and the hybrids, which also resulted in a lower number of flowers (Fig. 5).

In the *Oe. aquatica* habitat, mortality was highest in the summer when plants started flowering. The majority of deaths in the *Oe. aquatica* habitat occurred largely at the same time in both the hybrid and the two species and was caused by grazing by insect larvae (E. Westberg, pers. observ.). The insects fed on developing umbels so that even when plants managed to survive to flowering, their reproductive fitness was influenced. Mortality was highest in *Oe. coniooides* and F_1 hybrids, and among the survivors, the number of flowers was higher in *Oe. aquatica* than in the F_1 hybrid and *Oe. coniooides*. Grazing by snails and insect larvae was not observed at the *Oe. coniooides* site, although it was

repeatedly seen in natural populations of *Oe. aquatica* at other sites (E. Westberg, pers. observ.). The observed grazing by water-fowl at the *Oe. coniooides* site did not coincide with any significant increase of mortality.

Taken together, we hypothesize that improved tolerance to cold and wind exposure had to be acquired to be able to colonize the habitat of *Oe. coniooides*. At the same time, selection pressure for tolerance or defence against snail and insect grazing appears to be absent from the *Oe. coniooides* habitat. Here, twice-daily inundation may severely limit the size of herbivore populations. Defence against herbivory is considered costly for plants (Purrington, 2000), and selection against the maintenance of high levels of defence chemicals in *Oe. coniooides* might be expected. This in turn could explain the observation that in the *Oe. aquatica* habitat the alien *Oe. coniooides* was much more affected by grazing than the native *Oe. aquatica*. These hypotheses, however, require further testing, and, unfortunately, nothing is known about the quality and quantity of secondary compounds of the two species or about the quality and quantity of herbivore populations in the two habitats.

A recent study showed that seed germination requirements differ between the two species, and this was interpreted as adaptation to their respective habitats (Jensch & Poschlod, 2008). Accordingly, selection against immigrants at the germination and seedling establishment stage may have a large effect on fitness, although this was not the subject of the present study.

Reproductive isolation may be furthered by additional mechanisms. First, habitat specialization results in ecogeographical isolation, which may well cause less movement of pollinating insects between the two habitats. Second, there appears to be a differentiation of flowering time. In nature, *Oe. coniooides* flowers over a much shorter period than *Oe. aquatica*, of which flowering individuals can be found over several months (Schmeil & Fitschen, 2003). This will limit the temporal opportunity for interspecific hybridization. Because this would restrict the formation of interspecific hybrids, divergence of flowering may be a very important factor of reproductive isolation in the *Oe. coniooides* habitat where selection was stronger against immigrants than against hybrids (Nosil *et al.*, 2005).

OENANTHE CONIOIDES: A CASE OF SYMPATRIC SPECIATION?

The distribution area of *Oe. coniooides* is situated well within the geographical range of *Oe. aquatica*. In combination with the finding that *Oe. coniooides* is a young taxon (based on the absence of ITS divergence between the two species), Kadereit & Kadereit (2005) suggested the possibility of an *in situ* sympatric origin

of *Oe. coniooides*. The adaptive differentiation between the two species found in the present study, and the potential mechanisms of reproductive isolation discussed above, are not inconsistent with this possibility, especially if the habitat-shift of *Oe. coniooides* led to ecogeographical and/or temporal isolation. On the other hand, the extant distribution of the two species need not have been the same at the time of divergence (Losos & Glor, 2003), especially if *Oe. coniooides* originated earlier than the Holocene. During the last glacial maximum, the Scandinavian ice sheet extended close to the modern distribution area of *Oe. coniooides* (Lang, 1994), and growth of both species in a periglacial environment appears unlikely considering their ecology. In addition, lower sea levels during glacial periods most likely would have displaced the area of fresh water tides downstream. In summary, essentially nothing is known about the place of origin of *Oe. coniooides*. Dating the split between the two taxa could provide useful information on this question, and the finding of a Holocene origin of *Oe. coniooides* would make its *in situ* sympatric origin from *Oe. aquatica* more likely.

ACKNOWLEDGEMENTS

We are grateful to H. Kurz, H. Bertram, and H.-H. Hentschel for providing help and information during the collection of seeds and plant material; H. Below for advice on the cultivation of plants; W. Prott for permission to conduct transplantation experiments; J. Martens, H. Köpke, and H. Bertram for help with finding experimental sites; H. Kurz for helpful discussions; and C. Geyer for advice on the ASTER analyses. Three anonymous reviewers have provided helpful comments on earlier versions of this paper. The project was funded by DFG grant KA 635/12-1 to J. W. Kadereit and H.-H. Poppendieck.

REFERENCES

- Arnold ML. 1997.** *Natural hybridization and evolution*. New York, NY: Oxford University Press.
- Barracough TG, Vogler AP. 2000.** Detecting the geographical pattern of speciation from species-level phylogenies. *The American Naturalist* **155**: 419–434.
- Below H. 1999.** Der Schierlings-Wasserfenchel (*Oenanthe coniooides*) – Anmerkungen zur Ökologie und Biologie einer endemischen Pflanzenart der Tideelbe. In: Härdtle W, ed. *Die Elbtalaue – Geschichte, Schutz und Entwicklung einer Flusslandschaft*. Lüneburg: Festschrift Prof. Amelung, 137–144.
- Below H, Hobohm C. 1998.** Fahrwasservertiefungen in der Tideelbe und mögliche Auswirkungen auf den Bestand des Schierlings-Wasserfenchel (*Oenanthe coniooides*). *Jahrbuch des Naturwissenschaftlichen Vereins für das Fürstentum Lüneburg e.V.* **41**: 103–115.
- Below H, Poppendieck HH, Hobohm C. 1996.** Verbreitung und Vergesellschaftung von *Oenanthe coniooides* (Nolte) Lange im Tiedegebiet der Elbe. *Tuexenia* **16**: 299–310.
- Bolnick DI, Fitzpatrick B. 2007.** Sympatric speciation: theory and empirical data. *Annual Review of Ecology Evolution and Systematics* **38**: 459–487.
- Bonin A, Bellemain E, Eidesen PB, Pompanon F, Brochmann C, Taberlet P. 2004.** How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* **13**: 3261–3273.
- Corander J, Waldmann P, Sillanpää MJ. 2003.** Bayesian analysis of genetic differentiation between populations. *Genetics* **163**: 367–374.
- Corander J, Marttinen P, Mäntyniemi S. 2006.** Bayesian identification of stock mixtures from molecular marker data. *Fishery Bulletin* **104**: 550–558.
- Coyne JA, Orr HA. 1997.** 'Patterns of speciation in *Drosophila*' revisited. *Evolution* **51**: 295–303.
- Coyne JA, Orr HA. 2004.** *Speciation*. Sunderland, MA: Sinauer Associates.
- Edmands S. 1999.** Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**: 1757–1768.
- Excoffier L, Smouse PE, Quattro JM. 1992.** Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
- Geyer CJ, Wagenius S, Shaw RG. 2007.** Aster Models for Life History Analysis. *Biometrika* **94**: 415–426.
- Hroudová Z, Zákavský P, Hroudá L, Ostrý I. 1992.** *Oenanthe aquatica* (L) Poir: seed reproduction, population structure, habitat conditions and distribution in Czechoslovakia. *Folia Geobotanica et Phytotaxonomica* **27**: 301–335.
- Ingvarsson PK, Whitlock MC. 2000.** Heterosis increases the effective migration rate. *Proceedings of the Royal Society of London Series B, Biological Sciences* **267**: 1321–1326.
- Jensch D, Poschlod P. 2008.** Germination ecology of two closely related taxa in the genus *Oenanthe*: fine tuning for the habitat? *Aquatic Botany* **89**: 345–351.
- Junge P. 1912.** Über die Verbreitung von *Oenanthe coniooides* (Nolte) Garcke im Gebiet der Unterelbe. *Jahrbuch Hamburgischen Wissenschaftlichen anstalten* **19** (3. Beih): 123–128.
- Kadereit G, Kadereit JW. 2005.** Phylogenetic relationships, evolutionary origin, taxonomic status, and genetic structure of the endangered local Lower Elbe river (Germany) endemic *Oenanthe coniooides* (Nolte ex Rchb.f.) Lange (Apiaceae): ITS and AFLP evidence. *Flora* **200**: 15–29.
- Kawecki TJ, Ebert D. 2004.** Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.
- Kropf M, Kadereit JW, Comes HP. 2003.** Differential cycles of range contraction and expansion in European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). *Molecular Ecology* **12**: 931–949.

- Lang G. 1994.** *Quartäre Vegetationsgeschichte Europas*. Jena: G. Fischer.
- Losos JB, Glor RE. 2003.** Phylogenetic comparative methods and the geography of speciation. *Trends in Ecology and Evolution* **18**: 220–227.
- Ludwig G, Schnittler M, eds. 1996.** *Rote Liste gefährdeter Pflanzen Deutschlands*. Münster: Landwirtschaftsverlag, 427–480.
- Mantel N. 1967.** The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.
- Meyer FH. 1957.** Über Wasser- und Stickstoffhaushalt der Röhrichte und Wiesen im Elballuvium bei Hamburg. *Mitteilungen aus dem Staatsinstitut für Allgemeine Botanik in Hamburg* **11**: 137–203.
- Nosil P, Vines TH, Funk DJ. 2005.** Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**: 705–719.
- Nybom H. 2004.** Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.
- Peakall R, Smouse PE. 2006.** GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288–295.
- Purrrington CB. 2000.** Costs of resistance. *Current Opinion in Plant Biology* **3**: 305–308.
- Rieseberg LH, Baird SJE, Gardner K. 2000.** Hybridization, introgression, and linkage evolution. *Plant Molecular Biology* **42**: 205–224.
- Rousset F. 1997.** Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.
- Schluter D. 2000.** *The ecology of adaptive radiation*. New York, NY: Oxford University Press.
- Schmeil O, Fitschen J. 2003.** *Flora von Deutschland und angrenzender Länder*. In: Senghas K, Seybold S, eds, (91. ed.), Wiebelsheim: Quelle & Meyer.
- Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M. 1995.** AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407–4414.
- Walter HS, Gillet HJ, eds. 1998.** *1997 IUCN red list of threatened plants*. Compiled by the World Conservation Monitoring Centre IUCN. Gland: The World Conservation Union.
- Whitlock MC, Ingvarsson PK, Hatfield T. 2000.** Local drift load and the heterosis of interconnected populations. *Heredity* **84**: 452–457.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Average width of the largest leaf in the *Oenanthe conioides* habitat. Error bars indicate one standard error.

Figure S2. Mean number of rosette leaves per plant in the *Oenanthe conioides* habitat. Error bars indicate one standard error.

Figure S3. Average width of the largest leaf in the *Oenanthe aquatica* habitat. Error bars indicate one standard error.

Figure S4. Mean number of rosette leaves per plant in the *Oenanthe aquatica* habitat. Error bars indicate one standard error.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.