doi:10.1111/j.1558-5646.2011.01222.x

INFERENCE OF SELECTION AND STOCHASTIC EFFECTS IN THE HOUSE MOUSE HYBRID ZONE

Petra Dufková,[1,2,3,4] Miloš Macholán,[5] and Jaroslav Piálek[1,3]

[1]*Institute of Vertebrate Biology, Academy of Science of the Czech Republic, Brno, Czech Republic*

[2]*Department of Genetics, University of South Bohemia, České Budějovice, Czech Republic*

[3]*Department of Population Biology, Institute of Vertebrate Biology AS CR, Studenec 122, 67502, Koněšín, Czech Republic*

[4]*E-mail: dufkop@seznam.cz*

[5]*Laboratory of Mammalian Evolutionary Genetics, Institute of Animal Physiology and Genetics AS CR, Brno, Czech Republic*

Received July 21, 2010
Accepted December 14, 2010

We explored the transition of 13 X-linked markers across two separate portions of the house mouse hybrid zone, asking whether such a comparison can distinguish the effects of selection from random factors. A heuristic search in the likelihood landscape revealed more complex likelihood profiles for data sampled in two-dimensional (2D) space relative to data sampled along a linear transect. Randomized resampling of localities analyzed for individual loci showed that deletion of sites away from the zone center can decrease cline width estimates whereas deletion of sites close to the center can significantly increase the width estimates. Deleting localities for all loci resulted in wider clines if the number of samples from the center was limited. The results suggest that, given the great variation in width estimates resulting from inclusion/exclusion of sampling sites, the geographic sampling design is important in hybrid zone studies and that our inferences should take into account measures of uncertainty such as support intervals. The comparison of the two transects indicates cline widths are narrower for loci in the central part of the X chromosome, suggesting selection is stronger in this region and genetic incompatibilities may have at least partly common architecture in the house mouse hybrid zone.

KEY WORDS: **Cline analysis, *Mus musculus*, reproductive isolation, speciation, X chromosome.**

Identification of the genomic regions contributing to reproductive isolation is a fundamental key to understanding of speciation. Such studies require the detection of natural selection, teasing apart deterministic selective effects from stochastic effects and, ideally, identifying their causes. Although seemingly a trivial task, there are still very few datasets that can be used for the rigorous assessment of the effects of selection versus stochastic effects in natural populations.

Selection can be detected in natural populations if three conditions are met: variation exists in a trait under study, this variation is related to fitness differences among individuals, and it is at least partly heritable (Endler 1986). In a large population, these conditions are necessary for predicting the systematic change in gene frequencies over many generations; however, most natural populations consist of more or less locally isolated reproducing units with finite numbers of individuals. This geographical substructuring causes random perturbations from predicted gene frequencies (Wright 1969). These stochastic processes can decrease or increase selection estimates derived from observed gene frequencies, occasionally suggesting selection where there is not. Generally, it is difficult to delimit the effects of the deterministic and stochastic factors, especially as the latter can be a complex mixture of various processes either innate to the biological system studied (Millstein 2002), or introduced by investigators, for example during genotyping of samples (Pompanon et al. 2005).

Hybrid zones, defined as regions where genetically distinct populations meet, mate, and produce hybrids (Barton and Hewitt 1985), are natural laboratories for the study of the genetic basis of reproductive barriers, and are common in plants and animals (Harrison 1993; Ellstrand et al. 1996; Arnold 1997; Rieseberg and Willis 2007; Widmer et al. 2009). Tension zones are maintained by a balance between dispersal of parental genes into the zone and natural selection, which acts against hybrids or recombinants (Barton and Hewitt 1985; Barton and Gale 1993). For example, reduced fitness of hybrids or heterozygotes causes gradients of gene frequencies (clines) whose steepness is a function of the strength of selection (Haldane 1948; Slatkin 1973). Two extreme outcomes can be envisaged, depending on selection intensity. If hybrids are inviable or sterile, there will be no introgression at the hybrid zone center, and the cline width will approach zero. In contrary, a neutral gene will diffuse freely through the hybrid zone and the cline width will become infinity (Barton and Hewitt 1985). Because a variety of models of selection on single loci produce clines with similar shapes (Barton and Gale 1993; Kruuk et al. 1999), we can directly compare cline widths at different loci and thus determine genomic regions that can harbor genes involved in reproductive isolation. Indeed, many studies have documented differences in cline widths (Rieseberg et al. 1999; Marshall and Sites 2001; Martinsen et al. 2001; Sætre et al. 2003; Geraldes et al. 2006; Kronforst et al. 2006; Carling and Brumfield 2008, 2009; Carneiro et al. 2008; Stuckas et al. 2009; Teeter et al. 2008, 2010) with nearly 210-fold variation in the cline width estimates among markers (Carling and Brumfield 2009), suggesting suitability of this approach for detecting genetic incompatibilities between hybridizing taxa.

Studies of postzygotic reproductive barriers generally assume a model of speciation postulated by Bateson (1909), Dobzhansky (1937), and Muller (1942). According to the Bateson–Dobzhansky–Muller model, reproductive barriers can arise through accumulation of mutations in geographically separated populations, bringing about mutually incompatible combinations under secondary contact. This causes reduction of fitness in hybrid or recombinant individuals (Coyne and Orr 2004).

A review of *Drosophila* studies reveals that genes causing reproductive isolation are evolving rapidly, diverging as the result of positive selection (Orr 2005). This suggests these genes are fixed for alternative alleles in parental genomes. Under this assumption, reproductive barriers are expected to reduce gene flow at the same genomic regions along the whole length of a contact zone. Intuitively, a comparison of two or more geographically distant transects across the same hybrid zone can be used to distinguish the effects of selection and random processes. However, empirical studies measuring gene flow across different segments of the same hybrid zone in various organisms have come to conflicting conclusions: whereas in some cases, a consistent pattern across the transects has been reported (Szymura and Barton 1991; Buerkle and Rieseberg 2001; Gow et al. 2006), most studies have revealed incongruences between transects (Howard and Waring 1991; Cianchi et al. 2003; Vines et al. 2003; Riginos and Cunningham 2005; Gow et al. 2006; Yanchukov et al. 2006; Nolte et al. 2009; Aboim et al. 2010; Teeter et al. 2010). These incongruences could be due to drift or to heterogeneous selection. However, until now, no attempts to evaluate their effects have been made.

Here, we use multiple transect comparisons to explore the effects of deterministic and stochastic processes on cline shapes. To minimize the potential confounding effects of the environment, we chose the house mouse contact zone. Because this species lives predominately in and/or around human buildings, evolution of substantial ecological differences between hybridizing populations is unlikely (Sage 1981; but see Hunt and Selander 1973).

There are two house mouse subspecies in Europe: *Mus musculus musculus* (Mmm), which occupies eastern and northern Europe, and *M. m. domesticus* (Mmd), which occurs in western and southern parts of the continent. Along their contact, a ca. 2500 km long hybrid zone has been formed, transversing the Jutland Peninsula and running from the Baltic coast across Central Europe to the Black Sea (Boursot et al. 1993; Sage et al. 1993; Macholán et al. 2003; Fig. 1A). Genetic studies of this zone have been carried out in Denmark (Hunt and Selander 1973; Vanlerberghe et al. 1986, 1988b; Dod et al. 1993, 2005; Fel-Clair et al. 1998; Raufaste et al. 2005), East Holstein (Prager et al. 1993, 1997), the Czech Republic (Munclinger et al. 2002; Božíková et al. 2005, Macholán et al. 2007, 2008), Bavaria (Sage et al. 1986; Tucker et al. 1992; Payseur et al. 2004; Teeter et al. 2008, 2010), Saxony (Teeter et al. 2010), and Bulgaria (Vanlerberghe et al. 1986, 1988a). There is an agreement that this tension zone is maintained by selection on multiple genes (Raufaste et al. 2005; Macholán et al. 2007). Differential introgression has been reported between loci both within and among transects; however, a direct comparison among studies has been hampered by the application of different methods or sets of markers used in the analyses (see Macholán et al. 2007 for discussion). Despite these complicating issues, all studies collectively report significantly limited introgression of markers linked to sex chromosomes when compared to autosomal loci (Tucker et al. 1992; Dod et al. 1993; Macholán et al. 2007, 2008; Teeter et al. 2008, 2010). This conclusion is consistent with studies on various organisms, reporting a disproportionate involvement of sex chromosomes in speciation (Charlesworth et al. 1987; Coyne and Orr 1989; Sætre et al. 2003; Presgraves 2008; Qvarnström and Bailey 2009).

Despite reduced environmental influences in the mouse commensal habitat, there are several factors that can potentially have confounding effects on selection estimates. The most pervasive of these effects appears to be the biology of the species. House mice live in small reproductive units (demes) consisting usually of one

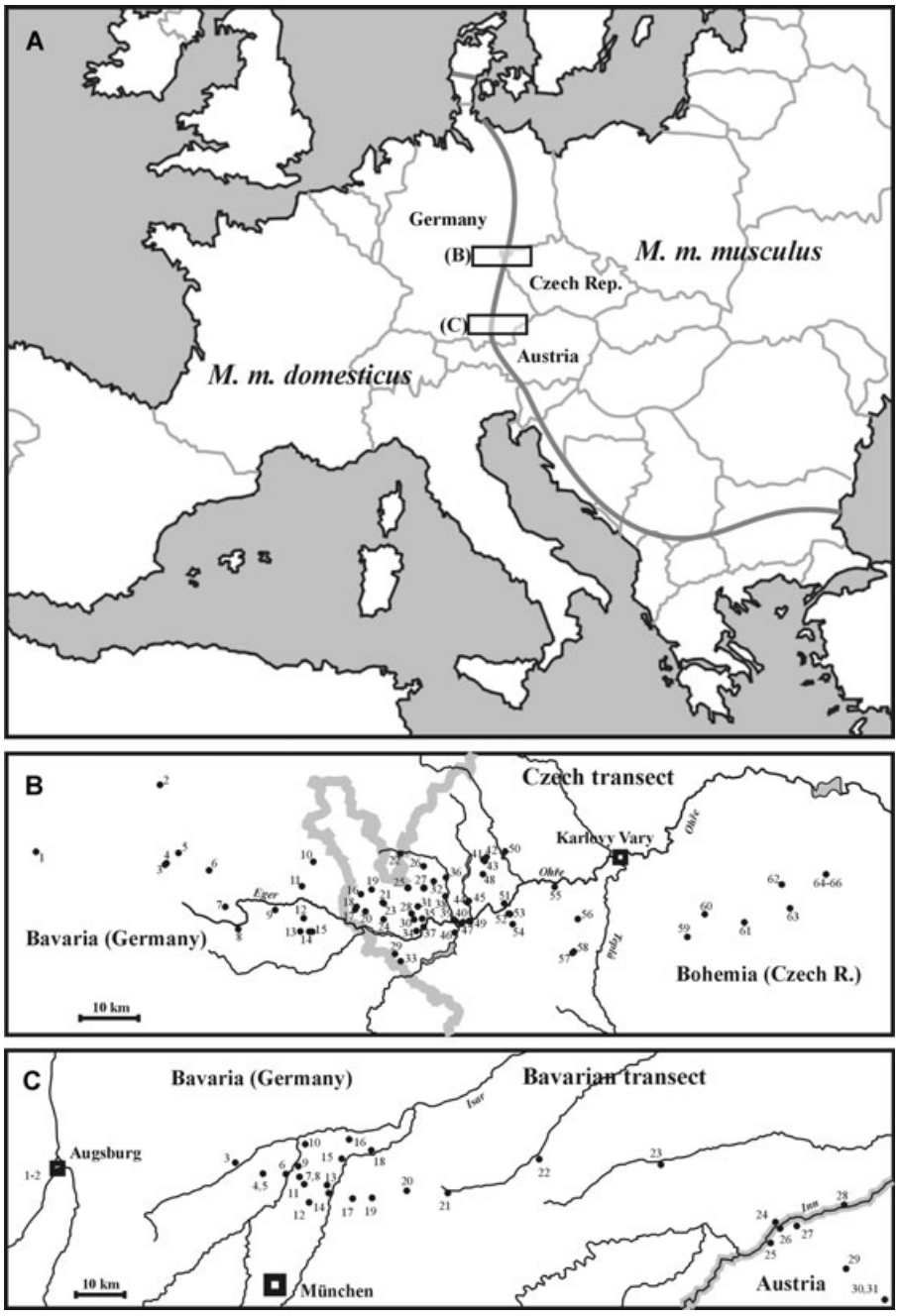

Figure 1. **A map of Europe showing the course of the house mouse hybrid zone with location of the two transects indicated by shaded rectangles (A) and positions of localities in the Czech (B) and southern Bavarian (C) transects. The distance between the two transects is approximately 200 km. Numbers of localities in the Czech transect correspond to Appendix 1, numbers in the Bavarian transects correspond to Payseur et al. (2004).**

dominant male and few females (Berry 1981; Sage 1981). The distribution of demes in the landscape is not uniform, being restricted primarily to human settlements, and demes are subjected to high extinction–recolonization rates (Sage 1981; Hauffe et al. 2000; Pocock et al. 2004). Young mice are often forced to migrate (Gerlach 1990; Krackow and König 2008); although most movements are between adjacent demes, long-distance migration due to human transportation is not negligible (Pocock et al. 2005). Another source of stochastic change in gene frequencies is larger scale temporal fluctuations in population densities. Because these frequencies are then typically sampled by tens of individuals from tens of localities, the stochastic effects of the sampling process may be considerable. Furthermore, the results may be biased due to various kinds of processing error such as sample labeling,

pipetting, and genotyping (Bonin et al. 2004; Pompanon et al. 2005); however, this source of error is rarely addressed in the hybrid zone literature.

Given the importance of sex chromosomes in speciation, we concentrated our attention on the X chromosome in this article. We used genotype data at 13 X-linked markers from a Czech portion of the hybrid zone and compared the inferred cline widths with those from a German transect (Payseur et al. 2004, 2005) using the same set of loci and analysis software. By standardizing the sets of loci and methods of analysis, we expected that if genetic incompatibilities were located in the same part of the chromosome, clines for loci located in this region would be as narrow in the Czech Republic as in Bavaria (Payseur et al. 2005). However, before a direct comparison, we wished to evaluate the stochastic factors that could affect gene frequencies at each locus and transect. We used jackknife resampling from the datasets and estimated the extent to which the output is prone to sampling perturbation. If the data were robust and converged on the same results then we would expect that selection is likely to operate on the loci under comparison. On the other hand, if the results showed high variation due to sampling perturbation we may conclude that inference on selective effects is overconfident. We asked three questions related to data robustness. What uncertainty and biases are introduced by the data handling and/or due to genotyping? Is the likelihood surface for the cline model uni- or multimodal? To what extent does sampling design affect the outcomes of parameter estimates?

Materials and Methods

SAMPLES

Czech transect

Mice were live-trapped at 66 localities within a 130-km long and 40-km wide rectangle located in western Bohemia (Czech Republic) and northeastern Bavaria (Germany) between 1991 and 2006 (hereinafter referred to as the Czech transect). Most animals were dissected in a field laboratory and approximately a third of live mice was transported each year to a breeding facility in Studenec for experimental breeding to assess fitness components of offspring. In the latter, parents were dissected in Studenec after completing the experiments and processed as their field-sacrificed counterparts. In total, 923 mice (505 males and 418 females) were analyzed. The sampling localities are listed in Appendix 1 and their positions within the transect are depicted in Figure 1B.

Bavarian transect

A total of 427 mice from 31 localities were collected by Richard D. Sage along a nearly 180-km-long linear transect stretched from southern Bavaria and western part of Upper Austria in 1984, 1985, and 1992. The positions of localities extracted from an erratum of Payseur et al. (2005) are shown in Figure 1C.

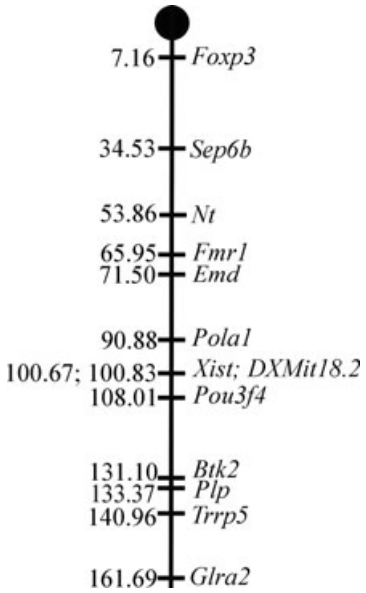

Figure 2. Studied loci and their position on the X chromosome (Mb) retrieved from NCBI Build 37.1 (http://www.ncbi.nlm.nih.gov). The filled circle indicates the centromere.

PANEL OF X-LINKED MARKERS

To avoid any inconsistency in transect comparison due to involvement of traits with different physical genetic position, we used the same panel of 13 subspecies-specific X-linked single nucleotide polymorphisms (SNPs) identified by Payseur et al. (2004; Fig. 2). SNPs from the Bavarian transect were genotyped in the laboratory of Michael W. Nachman at the University of Arizona using restriction fragment length polymorphism (RFLP) assays and were scored on 2% agarose gels (Payseur et al. 2004). For the Czech transect, DNA from ethanol-preserved spleens, livers, or tails was isolated using DNeasy® 96 Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). The three loci, *Glra2, Nt*, and *DXMit18.2*, were originally amplified through PCR and cut with restriction endonucleases. Restriction fragments were then scored on 2% agarose gels according to Payseur et al. (2004). Because the restriction enzymes used for scoring *Nt* and *DXMit18.2* did not cut all DNA fragments completely each time, we minimized the risk of false genotype assignment by replicating the numbers of runs. Later, an improved genotyping was achieved when we analyzed the two markers along with another 10 loci using an ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Carlsbad, CA) following a modified procedure of Kawałko et al. (2009). The SNPs were amplified using two separate hexaplex PCRs with the Multiplex PCR Kit (Qiagen). Each hexaplex was processed in 5-μl volume following manufacturer's instructions. Hexaplex I comprised *Foxp3* (final concentration of primers in reaction 0.2 μM), *Nt* (0.4 μM), *Fmr1* (0.5 μM), *Pola1* (0.5 μM), *DXMit18.2* (0.3 μM), and *Plp* (0.5 μM), with an annealing temperature of 58°C. Hexaplex II comprised *Sep6b* (0.6 μM), *Emd* (0.2 μM), *Xist*

(0.6 μM), *Pou3f4* (0.4 μM), *Btk2* (0.25 μM), and *Trrp5* (0.2 μM), with an annealing temperature of 62°C. After PCR amplification, both hexaplexes were mixed and purified by MinElute™ 96UF Purification Kit (Qiagen), subsequently used as templates for the second PCR (SNaPshot PCR) with unlabeled internal primers. SNaPshot primers, their concentrations in SNaPshot PCR (total volume 5 μl), and substitution types for Mmd or Mmm are described in Kawałko et al. (2009). The internal primer for *Nt* was 5′-$(GACT)_{11}$GTTGTTGGGTGATGTGGCCCT-3′ (final concentration 0.3 μM in SNaPshot PCR) and for *DXMit18.2* it was 5′-$(GACT)_6$GAAATTCTGTAAGTATAGATGAG-3′(0.2 μM). In both cases, subspecies-specific substitutions are A/G (*musculus*/*domesticus*). All 12 loci were separated in a single reaction using an ABI PRISM 3130 Genetic Analyser. Alleles were scored with Genemapper version 3.7 software (both Applied Biosystems).

ACCURACY OF GENOTYPE SCORES

As the two transects were analyzed using two different molecular techniques (RFLP vs. SNaPshot), it was necessary to test whether the two methods yielded comparable results. The *Nt* and *DXMit18.2* loci, identified as the markers most sensitive to restriction conditions, were chosen for this comparison. Genotyping errors can also be introduced in SNaPshot genotyping due to various human and nonhuman factors such as mutation in primers, DNA quality, sample manipulation, tube marking, pipetting, or subjectivity of allele scoring when stutters are present (Pompanon et al. 2005). Consequently, it was essential to estimate both types of errors, and to evaluate their impact on observed data.

Repeatability of the molecular methods and the accuracy of scored genotypes were analyzed using an independent dataset composed of 379 sons delivered in captivity by 159 wild-trapped parental pairs and hence with known pedigree. Male offspring were chosen to minimize the risk of reading error as they carry a single copy of the X chromosome, and the known maternal pedigree is sufficient for tracing parental alleles. DNA from ethanol-preserved spleens was isolated from the males as described above. The same SNaPshot™ Multiplex Kit method used for scoring wild mice (two hexaplexes without *Glra2*) was applied for genotyping the descendant males. The *Nt* and *DXMit18.2* loci were scored also with the same RFLP method used for wild mice. The scored genotypes of male offspring were compared to maternal genotypes and the proportion of mistyped alleles per locus and averaged over all loci was calculated for both datasets (i.e., genotyping error for females was estimated from genotype arrays of sons and vice versa).

CLINE ANALYSIS

Unlike the simple sampling design applied in the Bavarian transect (Sage et al. 1986; Payseur et al. 2004) following a straight line with westeast orientation, the Czech transect was sampled in two dimensions. The Cartesian coordinates were projected onto a one-dimensional (1D) axis parallel to the most likely direction of change in allele frequencies, estimated in Macholán et al. (2008), and ordered with increasing distance from the most western locality (Mmd). It should be noted that the Bavarian transect analysis has no such correction, and assumes that the cline widths are measured perpendicular to the hybrid zone.

Point estimates for cline shape parameters in the Bavarian transect were obtained using the ClineFit software (Porter et al. 1997; available at http://www-unix.oit.umass.edu/~aporter/software/). This likelihood-based program fits individual genotypic data to cline models. Because all but one locus analyzed in the Bavarian transect displayed asymmetric introgression (Payseur et al. 2004), we employed Barton's (1983) asymmetric stepped model based on six parameters (cline center, c; cline width, w; and four parameters describing introgression tails on either site of the cline θ_R; θ_L; z_R, z_L; Porter et al. 1997) as the best approximation of introgression for this transect. However, before utilizing this model as a proxy for both transects, we first asked whether introgression pattern in the Czech transect is similar to that in Bavaria. Consequently, we compared a sigmoid model based on fitting two parameters (c and w) to the asymmetric stepped model fitting six parameters. The sigmoid models performed significantly worse for 12 loci in the Czech transect; the values of likelihood ratio test ranged from $LR = 2(\ln LL_0 - \ln LL_1) = 15.0$ for *Pola1* to $LR = 109.4$ for *Btk2* ($P < 0.05$ for all loci). The only exception was *Sep6b*, for which increasing the number of parameters resulted in nonsignificant improvement of fit ($LR = 0.7$; $P = 0.95$). Given the similarity in introgression patterns detected in both transects, we used the six-parameter model in all subsequent analyses.

In addition to the procedure of Payseur et al. (2004), that used default values for all six parameters when exploring the likelihood surface, we employed a wider searching strategy. A common feature of likelihood-based methods is the danger of being entrapped on a local optimum during the maximum likelihood search (Swofford et al. 1996). With increasing the number of parameters in a model, the likelihood surface may become rather complicated, often with multiple peaks of similar likelihood. This makes inspection of likelihood profiles highly desirable (Macholán et al. 2007). However, this may be problematic with some software, especially in cases in which a higher number of loci are examined. Therefore, we explored a potential influence of multiple starting points on the maximum likelihood search. For this analysis, the cline width was chosen, starting with values of $w = 1$ km and $w = 100$ km in addition to the default value ($w \approx 25$ km); hereinafter, the default-width search is referred to as BMF (Basic Model Fit), and the manipulated searches as AMF1 and AMF100 (Alternate Model Fit), respectively. When the likelihood surface is unimodal

or the search algorithm successfully avoids local-optimum entrapment, all three models should converge to the same width estimates.

EFFECT OF SAMPLING

Obviously, only a subset of individuals can be sampled and analyzed from a hybrid zone. Consequently, cline parameter estimates can be influenced by the sampling design, which can be biased due to an observer's decisions influenced by geographical and physical limitations of the access to available localities and the presence or absence of individuals at localities inspected during a particular sampling period. The magnitude of this influence was tested using the following jackknife-based randomization procedure. For each locus, one locality at a time was deleted, keeping information on its identity. The remaining data were fitted with the six-parameter model using default values as described above. This procedure was repeated 100 times for each transect separately (Appendix 1; see also Table 1 in Payseur et al. (2004) for a list of localities along the Bavarian transect). The rationale of this method is that if systematic factors affecting the hybrid zone dynamics prevail, and both datasets are robust to the disturbing effects of the sampling design, we should expect the maximum likelihood estimates (MLEs) of the parameters to be clustered around the MLE conditioned on the full dataset. Based on the resulting width MLEs, the loci were classified as unimodal—with one main cluster (sometimes with one or a few outliers), or multimodal—with two or more clearly separated clusters. For each outlier, we inspected the lists of deleted localities to determine whether the result was caused by either the exclusion of a locality outside of the central part of the zone, or a site from the zone center. The central segment of the hybrid zone was defined arbitrarily as an area with localities falling into a belt ± 5 km from the cline center (Macholán et al. 2007).

Results

ACCURACY OF GENOTYPE SCORES

Inspection of genotypic data scored by the two molecular techniques in 379 individuals showed that genotyping error was 0.94% and 2.83% for *Nt* and *DXMit18.2*, respectively. In total, 11 false homozygotes were detected with RFLP. They were found to be either heterozygotes (10 cases) or a homozygote for the other allele.

For the quantification of an error rate for the SNPs dataset due to methodological or human errors, 4533 alleles were read at 12 loci using the SNaPshot multiplex in sons and 3816 alleles in mothers. The 28 alleles of these sons were incongruent with maternal genotypes. The overall error rate ranged from 0.00% (*Foxp3*, *Sep6b*, and *Btk2*) to 1.85% (*Fmr1*), with the average of 0.62%. In mothers, 17 genotypes were corrected, either from homozygote to heterozygote (15 changes) or from one homozygote to another one (2 changes). In total, 19 alleles were changed, resulting in the mean genotyping error to be 0.50%. The wild-trapped mothers formed a subset of the dataset used for cline width estimates and in all subsequent analyses we used their corrected genotypes.

GENOTYPES AT X-LINKED LOCI IN THE CZECH HYBRID ZONE

Individual genotypes scored at 1341 chromosomes and allele frequencies at 66 localities for the X-linked loci from the Czech transect are provided in Table S1 and Table S2, respectively. Scatterplots of distributions of the Mmm allele frequencies at scored loci along geographic distance are shown in Figure S1. These scatterplots indicate high variation in introgression patterns among loci and an asymmetry in gene flow at most loci. In general, no Mmm alleles were found within the westernmost sites between 0 and 45 km of the transect, except for rare occurrences of Mmm alleles at *Btk2* in Straas (22.5 km; $p_m = 0.05$) and *Sep6b* in Plössberg (45.6 km; $p_m = 0.25$). On the other hand, even in the eastern most village of Buškovice (126.6 km), Mmm alleles were not fixed at *Nt* and *Trrp5* with $p_m = 0.33$ and $p_m = 0.8$, respectively (Table S2). Minimal introgression was observed at the *Pou3f4* and *Emd* loci (Fig. S1).

TRANSECT COMPARISON: BASIC AND ALTERNATE MODEL FITS

Cline shape parameters for the studied loci estimated for the Czech transect and for the corrected dataset from the Bavarian transect (Payseur et al. 2005) are provided in the Table S3. A comparison of cline width estimates between the two transects based on BMF and AMF is shown in Figure 3A–C. Note the results presented for the Bavarian transect in Figure 3A are slightly different from those presented by Payseur et al. (2005). First, the cline widths are plotted against physical position of loci rather than their relative position in genetic map units (cM), which can largely depend on the crosses from which they are derived and may also differ between sexes (Shifman et al. 2006). Second, the *Plp* locus revealed consistently higher cline width estimates in our runs using BMF (cf. 14.90 km in Table S3 vs. 4.77 km reported by Payseur et al. 2005). For subsequent analyses, we will use the estimates derived in this study.

The BMF showed higher variation in cline widths among X-linked loci in the Czech transect (mean cline width $\bar{w} = 6.43$ km; range: from 0.43 km for *Pou3f4* to 25.02 km for *Sep6b*; coefficient of variation: $CV = 1.20$) when compared to the Bavarian transect ($\bar{w} = 3.93$ km; range: 0.57 for *Pola1* to 14.90 km for *Plp*; $CV = 0.97$).

When likelihood searches were started with cline width set to 1 km (AFM1), the results differed between the two datasets:

Table 1. Results of the jackknife analysis showing minimal and maximal values of cline widths (w_{min} and w_{max}) estimated from 100 replications. The jackknife values formed either one (1 cl) or two or more clusters (>1 cl). Many single clusters have one (1 out) or two or more isolated outliers (>1 out). The outliers appeared due to exclusion of a sampling site located at either of the edges (EHZ) or from the central segment (CSHZ) of the hybrid zone.

Czech transect									Bavarian transect							
Marker	w_{min}	w_{max}	1 cl	1 out	>1 out	EHZ	CSHZ	>1 cl	w_{min}	w_{max}	1 cl	1 out	>1 out	EHZ	CSHZ	>1 cl
Foxp3	0.80	10.79	x	x			x		2.66	6.03	x	x			x	
Sep6b	16.76	26.12						x	6.22	8.14	x					
Nt	0.41	3.62	x						1.03	6.63	x	x			x	
Fmr1	0.23	4.28	x	x			x		1.78	8.48						x
Emd	0.63	3.81	x	x			x		1.11	6.16	x	x			x	
Pola1	0.09	8.09						x	0.27	5.61	x	x			x	
Xist	7.48	16.77	x		x	x	x		1.59	2.76	x					
DXMit18.2	1.20	5.80	x	x			x		2.21	4.91	x	x			x	
Pou3f4	0.31	1.65	x						3.89	6.81	x					
Btk2	1.04	3.10	x						1.00	8.50						x
Plp	1.36	2.47	x						4.73	17.03						x
Trrp5	0.37	4.69	x	x			x		1.45	5.36	x	x			x	
Glra2	1.15	8.44						x	1.78	4.34	x					

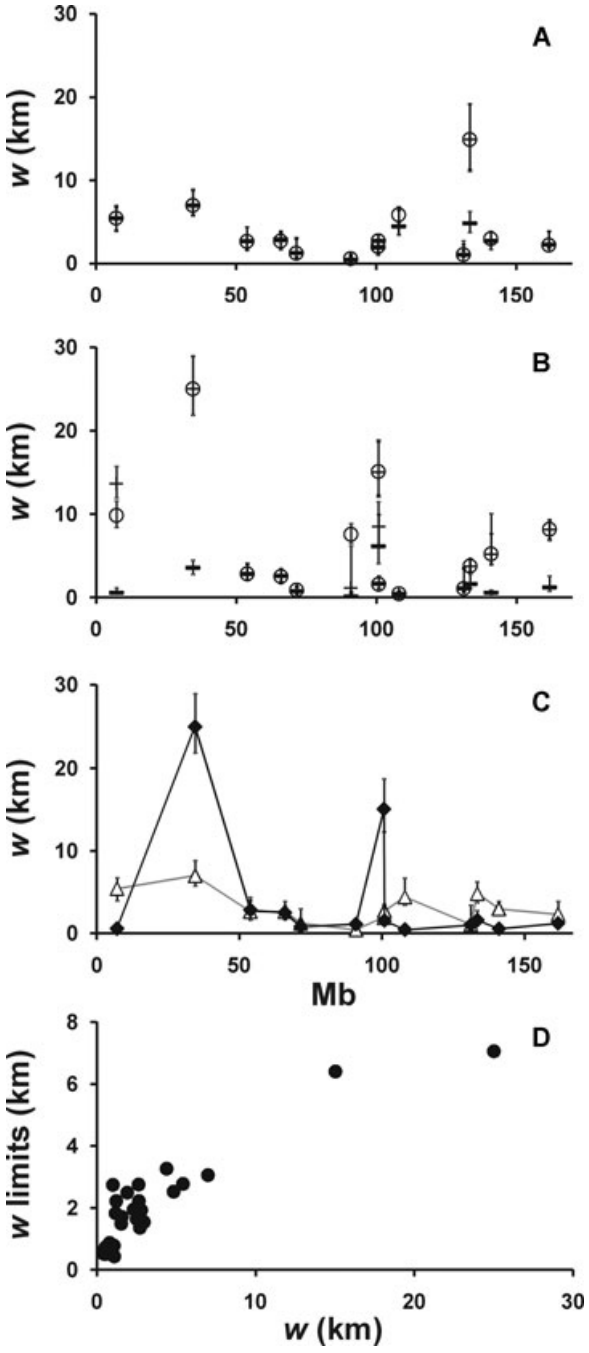

Figure 3. Estimates of cline widths (w) with two-unit support limits at 13 studied loci plotted against their physical position along the X chromosome for the Bavarian (A) and Czech (B) transects. The estimates were obtained with BMF (circles), AMF1 (– sign), and AMF100 searching scenarios (+ sign; see Material and Methods for their description). (C) The best maximum likelihood estimates detected from the three scenarios plotted separately in A and B. The lines connecting the point estimates are drawn for better orientation and have no predictive power. (D) Differences between the lower and upper values of two-unit support limits for widths plotted in (C) with extracted information about transects.

although nearly fourfold decrease in the average value was observed in the Czech transect ($\bar{w} = 1.76$ km; range: 0.10 km for *Pola1* to 6.12 km for *Xist*; $CV = 0.95$; see Table S3), in the Bavarian transect only cline width for *Plp* locus dropped from 14.90 to 4.83 km (Fig. 3A and Table S3), the value consistent with the estimate reported by Payseur et al. (2005). This drop in *Plp* decreased the average cline width to $\bar{w} = 3.05$ km (range: 0.44–7.0 km; $CV = 0.62$). One locus, *Xist*, was found to be wider in the Czech transect, seven loci had the same width in both transects, and five were wider in the Bavarian transect (cf. Fig. 3A and B).

In the AMF100 analyses with cline width initiated at 100 km, the estimates of cline widths converged mostly to the values estimated with BMF. For the Czech transect, the average cline was slightly wider than in the BMF and AMF1 strategies ($\bar{w} =$ 6.78 km, range: 0.44–25.02 km; $CV = 1.08$; Table S3). This difference was due to the most proximal locus, *Foxp3*, exceeding the BMF value (13.65 vs. 9.81 km). On average, the cline widths were also not different from the previous estimates in the Bavarian transect ($\bar{w} = 3.81$ km, range: 0.46–14.9 km; $CV = 1.00$; Table S3).

Summarizing the three fitting scenarios, we found higher consistency in cline width estimates in the Bavarian transect. Here, all but one marker converged to similar values with overlapping two-unit intervals. The only exception was the locus *Plp* reaching a maximum $w = 14.9$ km in BMF and AMF100 (maximum Log-Likelihood (LL) $= -137.13$) and a minimum $w = 4.83$ in AMF1 ($LL = -134.69$). In the Czech transect, only five loci (*Nt*, *Fmr1*, *Emd*, *Pou3f4*, and *Btk2*) did not differ between the three fitting scenarios. Seven loci yielded two similar width estimates and differed in one value, mostly produced by AMF1. Finally, for *Foxp3*, three different point estimates for cline width were found (BMF: $w = 9.81$, $LL = -273.68$; AMF1: $w = 0.54$, $LL = -262.28$; AMF100: $w = 13.65$, $LL = -277.20$).

The best MLEs derived from the three fitting methods are depicted in Figure 3C. In both transects, the width increases from the centromere to the *Sep6b* locus (34.5 Mb) where it reaches the highest value both in the Czech ($w = 25.02$ km; two-unit support limits 21.87–28.93) and Bavarian transects ($w = 7.00$ km; 5.76–8.82). Toward the middle part of the chromosome, the widths decrease consistently in both the Czech and Bavarian transect between 54 and 91 Mb (i.e., between *Nt* and *Pola1*). Further distally, the widths vary among loci but remain relatively low except of *Xist* (100.7 Mb), which reveals a seven times wider cline in the Czech transect ($w = 15.03$ km) relative to the Bavarian transect ($w = 1.92$ km). The narrowest cline was revealed for *Pola1* (90.9 Mb) in both transects (Czech: $w = 0.10$ km, support limits: 0.06–0.50; Bavarian: 0.44 km, 0.24–0.87).

Stochastic effects introduce uncertainty into the point estimates of cline width. One measure of uncertainty is the range of

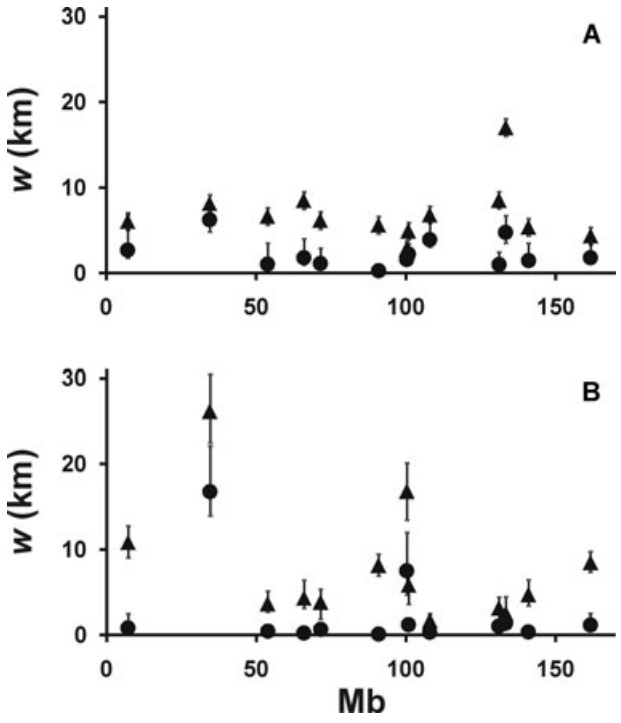

Figure 4. The jackknife cline width estimates showing the lowest (circles) and highest (triangles) values with two-unit limit support over 13 X-linked loci. (A) Bavarian transect, (B) Czech transect.

the two-unit support limits around the MLE. We detected a significant positive relationship between cline width estimates and their support limits for both transects (Fig. 3D). The relationship was best modeled as a nonlinear fit (two-unit support limits $\sim 1.5 \times w^{1/2}$) and explained the proportion of the variation at $R^2 = 0.96$ in the Czech and $R^2 = 0.54$ in the Bavarian transect, respectively ($P < 0.004$ in both cases).

TRANSECT COMPARISON: JACKKNIFE ANALYSIS

The estimates of cline widths from the 100 random replicates in both transects are shown in Figure S2. In comparison with the results obtained on the whole datasets, deletion of a locality resulted in finding wider intervals between minimum and maximum cline width estimates for all loci in the Bavarian transect, and eight loci in the Czech transect (Fig. S2). Seven loci (54%) in the Bavarian transect revealed nonoverlapping support limits between the lowest and highest estimate, whereas in the Czech transect it was as many as 11 loci (85%) (Fig. 4). The lowest estimates were due to deletion of samples mostly from the central segment of the hybrid zone. Only in four and two loci in the Bavarian and Czech transects, respectively, they resulted from exclusion of samples outside of this region. Conversely, the highest jackknife estimates were obtained exclusively after removing localities from the central segment of the hybrid zone. The highest relative differences between minimum and maximum cline widths were obtained in both transects for *Pola1*, the ratio w_{max}/w_{min} being 20.8 for the Bavarian and 89.9 for the Czech transect, respectively.

Table 1 summarizes the patterns of the distribution of cline widths due to a locality exclusion and this pattern is visualized in Figure S2. In both transects, 10 loci were centered around one cluster and three loci were more scattered, forming up to three separated clusters (*Sep3b* and *Glra2* in the Czech transect). In the former sort of loci, a prevailing feature was that outliers were found around the main cluster. These outliers were found in 12 of 20 cases caused by deletion of localities from the central segment of the hybrid zone, and occupied the space with higher cline width estimates. The only outlier resulting from the exclusion of a locality outside the zone center (*Xist* in the Czech transect) approached the lower limit of estimates of cline widths. As an example, Figure 5 gives the results of the jackknife analysis for the locus *DXMit18.2* in the Czech transect. Excluding the locality Milhostov1 (distance 68.6 km, about 0.3 km east of the consensus zone center), the analyses yielded two distinct outliers with a nearly fivefold increased width estimate.

It should be noted that the contribution of individual excluded localities to extreme values was not random. In the Bavarian transect, the sampling site with the most undue effect on the results was Neufahrn, deletion of which resulted in one the lowest, and seven the highest estimates. In the Czech transect, it was Dolnice rendering two the lowest, and five the highest estimates. Both sites are located within the central segment of the hybrid zone. To evaluate the effects of deletion in these localities upon cline widths for all 13 markers along the X chromosome, we ran the analyses using genotype arrays with Neufahrn and Dolnice, excluded from the datasets. As shown in Figure 6, the resulting estimates were more affected in the Bavarian transect than in the Czech transect.

Discussion

Manipulating both methodological procedures and sampling design, we tried to get an insight into a wide spectrum of stochastic effects that might potentially confound the hybrid zone analyses, consequently producing overconfident conclusions. The study was motivated by the idea that the partition of stochastic effects from selection affecting loci can be detected by comparison of cline shapes at two or more different transects (Szymura and Barton 1991; Buerkle and Rieseberg 2001; Nolte et al. 2009). To explore the relevance of this proposal, we compared two separate transects of the house mouse hybrid zone in Central Europe for the same set of 13 diagnostic X-linked markers described by Payseur et al. (2004, 2005). We applied three starting scenarios to cline width estimation to explore robustness of searches on the likelihood surface and analyzed data robustness by resampling localities. As genotyping of large datasets is subject to error, we found it necessary to estimate its extent.

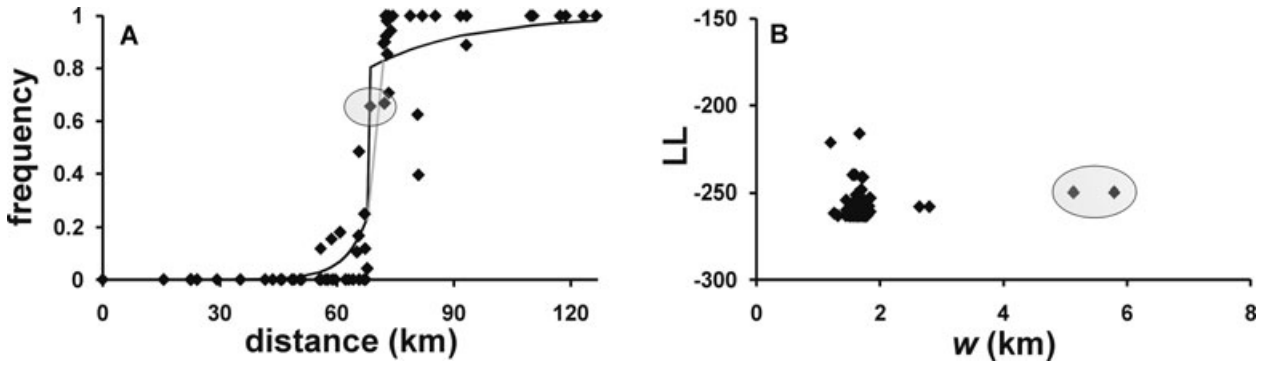

Figure 5. Jackknife analysis of cline widths for the locus *DxMit18.2* in the Czech transect. (A) Distribution of *musculus* allele frequencies along geographic distance with the cline fitted using the Basic Model Fit scenario for the whole dataset (solid line) and that fitted after excluding the locality Milhostov (dashed line). The position of Milhostov within the transect is indicated by a circle. (B) Deletion of Milhostov (distance 68.64 km, $p_m = 0.66$) resulted in an increase of the width estimates to 5.1 and 5.8 km (ellipse).

DATA ACCURACY

Reproducibility of the two molecular techniques applied in this work was rather high: 97.2% in RFLP and 99.1% in a technique using the SNaPshot™ Multiplex Kit for the *DXMit18.2* and *Nt* SNP markers. The mean error rate over both loci (1.89%) is comparable with the values reported for AFLP datasets derived for animal and plant species reaching 2.0–2.6%; Bonin et al. (2004). The vast majority of error reads detected in this study led to the correction of homozygotes to heterozygotes whereas in just one case one homozygote had to be replaced by the other homozygote. In the former case, the errors presumably arose as the result of the improper cutting activity of the restriction enzyme, whereas in the latter as the result of a pipetting error.

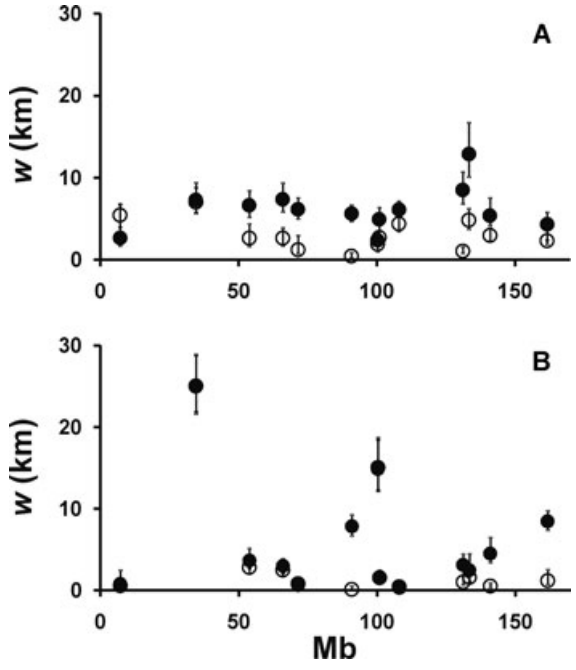

Figure 6. Comparison of cline widths for all 13 loci (open circles) with values estimated from the reduced datasets in which Neufahrn in the Bavarian transect (A) and Dolnice in the Czech transect (B) were excluded (closed circles). All estimates are given with two-unit support limits.

The mean error rate per locus was 0.56% for the SNP data, that is, about three times lower than that for the RFLP data. As with RFLP, most errors can be ascribed to difficulties in reading heterozygous genotypes due to the presence of stutter peaks. The allele calling error rate was locus specific: although most loci had little confounding effect on the observed data, *Nt* and *Fmr1* revealed 1.58% and 1.85%, respectively, almost reaching the error rates characteristic for RFLP. The presence of genotyping error may lead to erroneous inferences regarding population genetic estimates (see Pompanon et al. 2005 for review). For example, even error rates as low as 3% can have important ramifications in linkage disequilibrium measures (Akey et al. 2001). However, no such quantitative records could be retrieved for cline parameter estimates. To get an insight into the effect of genotyping error on cline width estimates, we used a genotype array for the *Plp* locus of the sons delivered in captivity ($N = 379$, see Materials and Methods). The reason for choosing this dataset was the requirement to employ data in which we could eliminate misidentified allele reads due to comparison of genotypes between mothers and their sons. The corrected dataset was fitted with BMF to get cline width point estimate for zero genotyping error. The dataset was then manipulated to model genotyping error up to 4% with the 0.5% increment. In each step, we changed randomly two new genotypes, and kept previously misread alleles unchanged. Despite some limitations of this approach, three points relevant to hybrid zone studies have emerged from the analysis (Fig. 7). First, the higher genotyping error the wider cline width point estimates. Second, this relationship might be at least in some cases nonlinear. As can be seen in Figure 7, two misread genotypes increased dramatically cline width point MLEs from 2.6 to 9.4 km. Third,

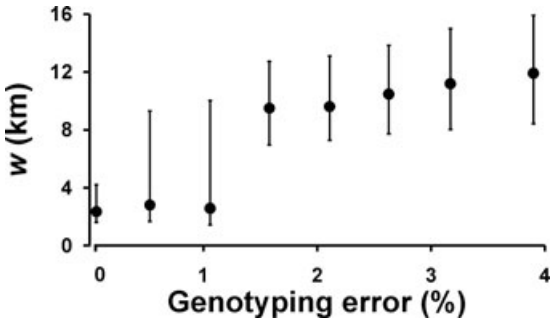

Figure 7. The relationship between genotyping error and cline width point estimates (w). Two-unit support limits for w are plotted. The increase for each genotyping error was achieved by changing two alleles in a genotypic array of 379 scored males.

point estimates can be flawed when genotyping error is as low as 1.6%.

All markers in the Bavarian transect were scored using RFLP and hence more prone to genotyping error. However, as our estimates of the RFLP genotyping error was performed in other laboratory it is difficult to evaluate its effect on cline width estimates, especially as there is no note about cutting efficacy of restriction enzymes (Payseur et al. 2004). The SNaPshot technique, used to score all SNP loci in the Czech transect, appeared to be more accurate and about 15% of the loci were at the limiting value for genotyping error detected by us and could not therefore be affected by this type of error.

Cline Widths Based on the Whole Datasets

VARIATION OF CLINE WIDTH ESTIMATES IS RELATED TO CLINE WIDTH DIFFERENCES

Studies designed to detect barriers to gene flow in hybrid zones have been facilitated by the development of analytical frameworks (Haldane 1948; Slatkin 1973; Barton and Hewitt 1985; Szymura and Barton 1986; Barton and Gale 1993; Kruuk et al. 1999) implemented into programs for fitting various cline models to the observed data (Barton and Baird 1995; Porter et al. 1997; Gompert and Buerkle 2009, 2010). Lesser effort has been invested in the analyses of various confounding factors that could influence estimates of cline shape parameters and inferences about reproductive isolation. Felsenstein (1975) and Slatkin and Maruyama (1975) analyzed the effect of random drift in finite populations in the linear stepping-stone model of a hybrid zone in both haploid and diploid organisms. The linear models were later extended into two dimensions by Nichols (1989). Random drift was modeled by drawing a finite number of individuals from a deme and sampling was with replacement. The authors concluded that stochastic effects are a function of deme size, migration and selection, and that they tend to make a cline less steep than the deterministic selection/gene flow case (Felsenstein 1975; Slatkin and Maruyama 1975; Nichols 1989). The inverse of the maximum gradient is used in tension zones as a measure of a barrier to gene flow that is proportionally related to dispersal (σ) and selection ($w \approx \sigma/s^{1/2}$, Slatkin 1973; Barton and Hewitt 1985). Stochastic effects are expected to vary from locus to locus depending on selection strength (Bierne et al. 2003). Two-unit support limits generated by programs for maximum-likelihood cline fitting take into account binomial sampling error within each locality sample (Carling and Brumfield 2009). Our data are in agreement with these predictions as the two-unit support limits were larger in the wider clines (Fig. 3D) in each transect. This finding indicates that selection is weaker at the corresponding loci. Due to a negative relationship between selection and random drift, stochastic factors could increase the variation of allele frequencies, causing the widening of clines. It should be noted that these constructs speculate that the dispersal and deme size affect the studied loci uniformly. This is not an unrealistic assumption. The dispersal estimated independently of linkage disequilibrium and cline width estimates from a set of microsatellite loci, based on the isolation-by-distance model, in the Czech transect was $\sim$800 m$\cdot$gen$^{-1/2}$ (Macholán et al. 2007). Unfortunately, there are no data on dispersal in the Bavarian transect. Nevertheless, the estimate from the hybrid zone in Denmark reporting values between 500–800 m$\cdot$gen$^{-1/2}$ (Raufaste et al. 2005) suggests that the dispersal of mice will be roughly at the same level.

No reliable data are available for deme sizes. However, because of the strict hierarchical organization of demic structure in Mmd and Mmm, it is reasonable to expect that deme sizes will be dominated by this social interaction and kept to the low numbers estimated to—four to six individuals (Hauffe et al. 2000). As both deme size and dispersal seem to be similar among the two transects, neighborhood size affecting the contact zone dynamics will be also of the same magnitude.

Despite differences between the two transects, the central part of the X chromosome (between 71 and 91 Mb, and between 108 and 133 Mb) appears to harbor loci under strong selection, as suggested by extremely narrow clines (Table 1, Fig. 3). This result was reported also by other authors (Storchová et al. 2004; Payseur et al. 2005; Teeter et al. 2010; Macholán et al. 2011) and thus it appears that there is some evidence of common architecture of reproductive isolation between the house mouse subspecies.

THE PRESENCE OF LOCAL LIKELIHOOD MAXIMA

Changing the starting points in the parameter space affirmed that the heuristic search for a width MLE can result in entrapment at local-optima. As expected, this happened more often on complex likelihood surfaces. Interestingly, the complexity of the likelihood landscape increased with the dimensionality of the sampling area.

Although the search only converged to different peaks in a single locus in the linearly sampled Bavarian transect, as many as five loci revealed multiple peaks in the two-dimensional Czech transect (cf. Fig. 3A and 3B).

The greater failure rate of the heuristic search arises when the Czech data are collapsed onto a line because there is a much greater density of localities along the line relative to the width of the zone. This has two effects: (1) Because of higher sampling density, the likelihood model of within-locality sampling error is sufficient, and its support limits for width estimates explain 96% of the variation in jackknife estimates. This can be contrasted to the Bavarian case in which samples are originally taken along one line, resulting in a less-dense effective locality sampling. The within-locality sampling error is insufficient, and width estimate support is overconfident, as it only captures 46% of the variation in jackknife estimates. (2) The likelihood surface to be explored is more complex for dense localities on the line, requiring rigorous search techniques such as likelihood profiling (Phillips 2004; Macholán 2007) for avoidance of entrapment in local optima.

Another issue arises when the scale of sampling is large compared to the scale at which conditions change along the center of a hybrid zone—the course of the zone center might not be linear (Sites et al. 1995; Bridle et al. 2001) and/or the environment might change (MacCallum et al. 1998; Vines et al. 2003). The former scenario can be taken into account by likelihood-based fitting a nonlinear model of the cline center (Bridle et al. 2001), whereas the latter by allowing cline parameters to vary along the course of the center. The Czech sampling region spans at most 40 km north-south of the 2500 km long zone, so collapsing these onto a one-dimensional transect seems unlikely to confound centerline linearity (it should be recalled that the course of the zone was inferred from a much larger data, see Macholán et al. 2007, 2008).

However, this collapsing may bring together localities subject to uneven distribution of local physical barriers. As the result of the collapsing process, localities with different 2D contexts will be forced to occupy similar distances on the line adding an extra component of stochastic effects proportional to the difference in contexts. The complexity of allele frequency topology in space can also be increased by deviations from cline monotonicity due to either historical events (*Abpa* gene: Dod et al. 2005; mtDNA: Božíková et al. 2005), or single or joint effect(s) of selective advantage (as suggested by the data for the *Xist* locus in Fig. S1), ancestral polymorphisms (Geraldes et al. 2008; Čížková et al. 2011), hybrid zone movement (Macholán et al. 2011), or due to different behavior of 1D and 2D hybrid zones (Piálek and Barton 1997). As always, any gain in realism through increasing model complexity (e.g., 2D vs. 1D analysis) must be weighed against the loss of power arising from attempting to estimate more parameters.

Only sampling data in two dimensions has the power to reveal a nonlinear course of the cline center (Barton and Hewitt 1985; Bridle et al. 2001) or to detect a locus whose cline center has a different orientation from the consensual frequency change (Macholán et al. 2008). If such deviations from the parallel linear cline centers go undetected, collapsing data onto one dimension will result in systematically overestimation of cline widths (Macholán et al. 2008). On the other hand, where 2D sampling shows that 1D summary is appropriate, collapsing to 1D provides locality sampling density on the line sufficient to avoid overconfident likelihood estimates of cline width.

It should be noted that our results consider cline width exclusively. Including cline center estimation would add another dimension of complexity to our results, especially in loci that introgressed far away from the center of the hybrid zone (see the *Nt* and *Xist* loci in Fig. S1). Because the center and width are coestimated, a rigorous approach would involve reference to their joint 2D likelihood profile, an aspect left to future studies.

Cline Width Under Locality Resampling Datasets

In an ideal contact zone the allele frequencies change monotonically. If the underlying cline shape is close to that of the cline model, there will be a single peak on the likelihood surface and idealized likelihood searches should identify this one peak. With finite samples from natural hybrid zones, and nonidealized searching heuristics, multiple peaks can be detected as discussed above. One causal factor is the sampling design, depending on the effort invested into field work and also on availability of sites populated by animals. Below we discuss the effects of sampling design on cline width estimates using the locality resampling approach. Specifically, we ask for effects of locality resampling on cline width variation for each locus and how the sampling can affect the variation over the whole set of loci when one locality is not sampled.

PER LOCUS RANDOMIZATION

In both transects, nearly half of the analyses produced one cluster of similar cline width estimates, most of them having one or more outliers (Table 1, Fig. S2). The remaining loci were centered around two clusters although one locus displayed three different clusters (Fig. S2). This suggests that the sampling design is a pervasive factor affecting cline width estimates.

For the most part, deletion of localities from the central segment of the hybrid zone resulted in changes of cline width estimates. Barton and Gale (1993) emphasize that dense sampling near the center is vital for reliable estimation. We suggest that it is the interplay between the likelihood maximization heuristic and sampling design that explains this observation. As with the

Bavarian transects, the X-linked traits in the Czech transect were best fitted with six parameters allowing for asymmetric stepped clines (ClineFit, data not shown. The stepped model generally yielded estimates of cline center similar to that based on the sigmoid model, but as a rule it rendered narrower widths.). The asymmetry of gene flow in the Czech transect was already detected at five loci (two of them, *Nt* and *DXMit18.2*, also included in this study) (Macholán et al. 2007), and analyzed using the likelihood maximization heuristics in the Analyse program (Barton and Baird 1995). A comparison of the ClineFit and Analyse documented that both programs give congruent estimates of the cline center and width (Macholán et al. 2007). Collectively, these analyses suggest that X-linked loci in both transects are dominated by asymmetry in gene flow.

It appears that many loci might be under selection at the X chromosome with effective selection (s^*) being about 0.25 (Macholán et al. 2007). It is reasonable to expect that the same will be true for the Bavarian transect given the restricted introgression for the X chromosome markers relative to autosomal traits (Tucker et al. 1992; Teeter et al. 2008). But even for weak selection, multilocus clines can form a sharp step at their center due to associations across loci, whereas alleles behave independently in either flanking tail of introgression (Barton 1979, 1983; Barton and Gale 1993). Consequently, as cline width is estimated from the central segment of the hybrid zone, deletion of localities outside this part may have only a small effect on width estimates, whereas the exclusion of samples inside the central segment could lead to significantly different width estimates at individual loci. For example, we found point estimates to differ 21- and 90-fold times in the two transects as documented above for the *Pola1* locus (Fig. 4). From this point of view, dense sampling of localities from the central segment of a hybrid zone seems to be a necessity for studies trying to estimate cline shape and identifying chromosomal regions or genes under selection. However, it seems that the sampling density around the zone center is underappreciated in literature and only few studies mention its possible effect on cline parameter estimates. For example, sparse sampling of the central part of southern and northern transects of the *Ensatina* hybrid zone was stressed as a factor preventing the rigorous comparison (Alexandrino et al. 2005). Macholán et al. (2007) states explicitly that only upper bounds can be placed on width estimates when there is sparse central sampling relative to the width—another example where a point estimate without bounds is going to be misleading. More recently, unequal sampling design between the Saxony and Bavarian transects of the house mouse hybrid zone was noted among factors hampering the comparison of loci under study (Teeter et al. 2010).

The effects of locality resampling may be different when other cline models are assumed. For example, when gene flow is symmetric and not stepped, data can be fitted against an S-shaped sigmoid function (Haldane 1948; Szymura and Barton 1986, 1991; Porter et al. 1997; Kruuk et al. 1999). Then, the deletion of localities outside the central part of the hybrid zone may substantially change width estimates. For example, long-distance introgression of the Mmd mtDNA into the Mmm background was suggested in the Bavarian transect to explain unexpected frequencies at two localities about 90 km far from the center of the hybrid zone (Božíková et al. 2005). Over all localities the mtDNA cline width was calculated using a sigmoid model to be 100 km; when one and both mtDNA outlier localities were excluded, the width dropped to 62 and 24 km, respectively (Božíková et al. 2005).

PER SAMPLE RANDOMIZATION

Earlier we noted that cline width is mostly affected by the exclusion of samples from the zone center. We also mentioned that for the many loci we observed the presence of one or more outlier width estimates and/or two or three clusters of width estimates. As these outliers or multiple clusters bound the limits of our estimates of cline widths, we asked whether their distribution is random with respect to sample removal. We found that two localities disproportionally contributed to the presence of outliers, and increased cline widths for nearly 40% (Dolnice) and 55% (Neufahrn) in the Czech and Bavarian transect, respectively. In both cases the sampling localities are located in the central segment of the zone, 0.7–0.8 km from the consensus cline center. The higher effect of the Bavarian Neufahrn locality on cline parameters estimates can also be explained by the proportion to which this sample contributes in respect to the whole dataset. The locality is the second largest sample in the Bavarian transect and with 103 scored X chromosomes constitutes 15% of the whole genotypic array.

Comparison of the datasets comprising jackknife estimates, after deletion of the two localities and that of based on the best MLE (Table S3), suggested that in the Bavarian transect cline width estimates were affected due to deletion of Neufahrn in seven of 13 loci and concentrated in two regions of the X chromosome (54–91 and 131–141 Mb, Fig. 6A). No such consistent differentiation was observed in the Czech transect owing to Dolnice deletion, where cline width estimates were affected in three loci and only two of them were localized to the distal part of the chromosome (141–162 Mb, Fig. 6B).

The randomization techniques stress the importance of sampling design on observed data and how including/excluding one locality might affect the results. This importance is illuminated in Figure 4, which shows that the variation in cline width estimates at single loci due to one sample exclusion, reaching up to 84-fold increase, can be higher than the maximum of 50-fold variation in the widths among 39 SNP markers reported from the Bavarian transect by Teeter et al. (2008).

Concluding Remarks

The present work was initially motivated by the idea that the level of introgression measured via cline width is a convenient method to separate selected and neutral genes (see Payseur 2010 for review). However, soon it appeared such approach to be based on some idealistic assumptions and hence potentially misleading. On the other hand, the comparison of the Czech and Bavarian portions of the mouse hybrid zone proved to be a very fruitful tool for inferring selection and stochastic effects in hybrid zones. For example, consistently low estimates of cline width across the two transects for loci located at the central part of the X chromosome suggest strong counterselection in this region, showing that genetic incompatibilities between the two taxa may have at least partly common architecture.

However, our results indicate that searching for speciation genes through statistical analyses of cline shape parameters and their multiple comparisons is not a trivial task and is prone to overconfident conclusions. Genotyping error for SNP markers seems to have little effect on parameter estimates, and the advance of techniques of genome-wide genotyping (Yang et al. 2009) makes the effects of this type of error negligible. However, RFLP-based methods, which were more prone to genotyping error, can inflate cline width estimates. For future studies of hybrid zones, we recommend two-dimensional sampling as a necessity to ensure perpendicular cross-section orientation of geographic clines to the maximum gradient of genetic changes. The most intensive sampling should be focused on the central parts of hybrid zones because they disproportionally affect the estimates of cline widths.

Although we consider here the effects of sampling design on cline width estimates, Polechová and Barton (2011) re-explored the effect of finite-population sizes (i.e., random genetic drift). They found that random genetic drift makes the underlying clines on average steeper, relative to the deterministic case. As the clines due to genetic drift shift from side to side, averaging over many realizations (e.g., pooling data from distant generations), gives wider "expected" clines.

ACKNOWLEDGMENTS

O. Piálek wrote a program for the jackknife analysis. S. J. E. Baird, V. Pallazola, P. K. Tucker, and two anonymous reviewers provided valuable comments on earlier versions of the manuscript. The work was supported by the Czech Science Foundation (project 206/08/0640) and the Grant Agency of the Academy of Sciences of the Czech Republic (IAA600930506) to JP and MM, the Czech Ministry of Education (LC06073) to JP, and by SGA of Faculty of Science, University of South Bohemia and GAJU (137/2010/P) to PD.

LITERATURE CITED

Aboim, M. A., J. Mavarez, L. Bernatchez, and M. M. Coelho. 2010. Introgressive hybridization between two Iberian endemic cyprinid fish: a comparison between two independent hybrid zones. J. Evol. Biol. 23:817–828.

Akey, J. M., K. Zhang, M. Xiong, P. Doris, and L. Jin. 2001. The effect that genotyping errors have on the robustness of common linkage-disequilibrium measures. Am. J. Hum. Genet. 68:1447–1456.

Alexandrino, J., S. J. E. Baird, L. Lawson, J. R. Macey, C. Moritz, and D. B. Wake. 2005. Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. Evolution 59:1334–1347.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, New York.

Barton, N. H. 1979. Gene flow past a cline. Heredity 43:333–339.

———. 1983. Multilocus clines. Evolution 37:454–474.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Barton, N. H., and S. J. E. Baird. 1995. Analyse—An application for analyzing hybrid zones. Freeware, Edinburgh, U.K. Available at http://helios.bto.ed.ac.uk/evolgen/Mac/Analyse.

Bateson, W. 1909. Heredity and variation in modern lights. Pp. 85–101 *in* A. C. Seward, ed. Darwin and modern science, Cambridge Univ. Press, Cambridge.

Berry, R. J. 1981. Population dynamics of the house mouse. Symp. Zool. Soc. Lond. 47:395–425.

Bierne, N., C. Daguin, F. Bonhomme, P. David, and P. Borsa. 2003. Direct selection on allozymes is not required to explain heterogeneity among marker loci across a *Mytilus* hybrid zone. Mol. Ecol. 12:2505–2510.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13:3261–3273.

Boursot, P., J.-C. Auffray, J. Britton-Davidian, and F. Bonhomme. 1993. The evolution of house mice. Annu. Rev. Ecol. Syst. 24:119–152.

Božíková, E., P. Munclinger, K. C. Teeter, P. K. Tucker, M. Macholán, and J. Piálek. 2005. Mitochondrial DNA in the hybrid zone between *Mus musculus musculus* and *Mus musculus domesticus*: a comparison of two transect. Biol. J. Linn. Soc. 84:363–378.

Bridle, J. R., S. J. E. Baird, and R. K. Butlin. 2001. Spatial structure and habitat variation in a grasshopper hybrid zone. Evolution 55:1832–1843.

Buerkle, C. A., and L. H. Rieseberg. 2001. Low intraspecific variation for genomic isolation between hybridizing sunflower species. Evolution 55:684–691.

Carling, M. D., and R. T. Brumfield. 2008. Haldane's rule in an avian system: using cline theory and divergence population genetics to test for differential introgression of mitochondrial, autosomal, and sex-linked loci across the *Passerina* bunting hybrid zone. Evolution 62:2600–2615.

———. 2009. Speciation in *Passerina* buntings: introgression patterns of sex-linked loci identify a candidate gene region for reproductive isolation. Mol. Ecol. 18:834–847.

Carneiro, M., N. Ferrand, and M. W. Nachman. 2008. Recombination and speciation: loci near centromeres are more differentiated than loci near telomeres between subspecies of the European rabbit (*Oryctolagus cuniculus*). Genetics 181:593–606.

Cianchi, R., A. Ungaro, M. Marini, and L. Bullini. 2003. Differential patterns of hybridization and introgression between the swallowtails *Papilo machaon* and *P. hospiton* from Sardinia and Corsica islands (Lepidoptera, Papilionidae). Mol. Ecol. 12:1461–1471.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.

Čížková, D., J. Goüy de Bellocq, S. J. E. Baird, J. Piálek, and J. Bryja. 2011. Genetic structuring and different selection pattern at two major histocompatibility complex genes in wild house mouse

populations. Heredity advance online publication, September 8, 2010; doi:10.1038/hdy.2010.112.

Coyne, J. A., and H. A. Orr. 1989. Two rules of speciation. Pp. 180–207 *in* D. Otte and J. Endler, eds. Speciation and its consequences. Sinauer Associates, Inc., Sunderland, MA.

———. 2004. Speciation. Sinauer Associates, Inc., Sunderland, MA.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia Univ. Press, New York.

Dod, B., L. S. Jermiin, P. Boursot, V. H. Chapman, J. T. Nielsen, and F. Bonhomme. 1993. Counterselection on sex chromosomes in the *Mus musculus* European hybrid zone. J. Evol. Biol. 6:529–546.

Dod, B., C. Smadja, R. C. Karn, and P. Boursot. 2005. Testing for selection on the androgen-binding protein in the Danish mouse hybrid zone. Biol. J. Linn. Soc. 84:447–459.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proc. Natl. Acad. Sci. USA 93:5090–5093.

Endler, J. A. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.

Fel-Clair, F., J. Catalan, T. Lenormand, and J. Britton-Davidian. 1998. Centromeric incompatibilities in the hybrid zone between house mouse subspecies from Denmark: evidence from patterns of NOR activity. Evolution 52:592–603.

Felsenstein, J. 1975. Genetic drift in clines which are maintained by migration and natural selection. Genetics 81:191–207.

Geraldes, A., P. Basset, B. Gibson, K. L. Smith, B. Harr, H. Yu, N. Bulatova, Y. Ziv, and M. W. Nachman. 2008. Inferring the history of speciation in house mice from autosomal, X-linked, Y-linked and mitochondrial genes. Mol. Ecol. 17:5349–5363.

Geraldes, A., N. Ferrand, and M. W. Nachman. 2006. Contrasting patterns of introgression at X-linked loci across the hybrid zone between subspecies of the European rabbit (*Oryctolagus cuniculus*). Genetics 173:919–933.

Gerlach, G. 1990. Dispersal mechanisms in a captive wild house mouse population (*Mus domesticus* Rutty). Biol. J. Linn. Soc. 41:271–277.

Gompert, Z., and C. A. Buerkle. 2009. A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. Mol. Ecol. 18:1207–1224.

———. 2010. INTROGRESS: a software package for mapping components of isolation in hybrids. Mol. Ecol. Res. 10:378–384.

Gow, J. L., C. L. Peichel, and E. B. Taylor. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Mol. Ecol. 15:739–752.

Haldane, J. B. S. 1948. The theory of a cline. J. Genet. 48:277–284.

Harrison, R. G., ed. 1993. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Hauffe, H. C., J. Piálek, and J. B. Searle. 2000. The house mouse chromosomal hybrid zone in Valtellina (SO): a summary of past and present research. Hystrix 11:17–25.

Howard, D. J., and G. L. Waring. 1991. Topographic diversity, zone width, and the strength of reproductive isolation in a zone of overlap and hybridization. Evolution 45:1120–1135.

Hunt, W. G., and R. K. Selander. 1973. Biochemical genetics of hybridisation in European house mice. Heredity 31:11–33.

Kawałko, A., P. Dufková, J. M. Wójcik, and J. Piálek. 2009. Polymerase chain reaction multiplexing of microsatellites and single nucleotide polymorphism markers for quantitative trait loci mapping of wild house mice. Mol. Ecol. Res. 9:140–143.

Krackow, S., and B. König. 2008. Microsatellite length polymorphisms associated with dispersal-related agonistic onset in male wild house mice (*Mus musculus domesticus*). Behav. Ecol. Sociobiol. 62:813–820.

Kronforst, M. R., L. G. Young, L. M. Blume, and L. E. Gilbert. 2006. Multilocus analyses of admixture and introgression among hybridizing *Heliconius* butterflies. Evolution 60:1254–1268.

Kruuk, L. E. B., S. J. E. Baird, K. S. Gale, and N. H. Barton. 1999. A comparison of multilocus clines maintained by environmental adaptation or by selection against hybrids. Genetics 153:1959–1971.

MacCallum, C. J., B. Nürnberger, N. H. Barton, and J. M. Szymura. 1998. Habitat preference in the *Bombina* hybrid zone in Croatia. Evolution 52:227–239.

Macholán, M., B. Kryštufek, and V. Vohralík. 2003. The location of the *Mus musculus*/*M. domesticus* hybrid zone in the Balkan: clues from morphology. Acta Theriol. 48:177–188.

Macholán, M., P. Munclinger, M. Šugerková, P. Dufková, B. Bímová, E. Božíková, J. Zima, and J. Piálek. 2007. Genetic analysis of autosomal and X-linked markers across a mouse hybrid zone. Evolution 61:746–771.

Macholán, M., S. J. E. Baird, P. Munclinger, P. Dufková, B. Bímová, and J. Piálek. 2008. Genetic conflict outweighs heterogametic incompatibility in the mouse hybrid zone? BMC Evol. Biol. 8:271.

Macholán, M., S. J. E. Baird, P. Dufková, P. Munclinger, B. Vošlajerová Bímová, and J. Piálek. 2011. Assessing multilocus introgression patterns: A case study on the mouse X chromosome in Central Europe. Evolution, doi: 10.1111/j.1558-5646.2011.01228.x [Epub ahead of print].

Marshall, J. C., and J. W. Sites. 2001. A comparison of nuclear and mitochondrial cline shapes in a hybrid zone in the *Sceloporus grammicus* complex (Squamata; Phrynosomatidae). Mol. Ecol. 10:435–449.

Martinsen, G. D., T. G. Whitham, R. J. Turek, and P. Keim. 2001. Hybrid populations selectively filter gene introgression between species. Evolution 55:1325–1335.

Millstein, R. L. 2002. Are random drift and natural selection conceptually distinct? Biol. Philos. 17:33–53.

Muller, H. J. 1942. Isolating mechanisms, evolution, and temperature. Biol. Symp. 6:71–125.

Munclinger, P., E. Božíková, M. Šugerková, J. Piálek, and M. Macholán. 2002. Genetic variation in house mice (*Mus*, Muridae, Rodentia) from the Czech and Slovak Republic. Folia Zool. 51:81–92.

Nichols, R. A. 1989. The fragmentation of tension zones in sparsely populated areas. Am. Nat. 134:969–977.

Nolte, A. W., Z. Gompert, and C. A. Buerkle. 2009. Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. Mol. Ecol. 18:2615–2627.

Orr, H. A. 2005. The genetic basis of reproductive isolation: Insights from *Drosophila*. Proc. Natl. Acad. Sci. USA 102(Suppl.1):6522–6526.

Payseur, B. A. 2010. Using differential introgression in hybrid zones to identify genomic regions involved in speciation. Mol. Ecol. Res. 10:806–820.

Payseur, B. A., J. G. Krenz, and M. W. Nachman. 2004. Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mouse. Evolution 58:2064–2078.

———. 2005. Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mouse (vol 59, pg 474, 2005). Evolution 58:2064–2078.

Phillips, B. L., S. J. E. Baird, and C. Moritz. 2004. When vicars meet: a narrow contact zone between morphologically cryptic phylogeographic lineages of the rainforest skink, *Carlia rubrigularis*. Evolution 58:1536–1548.

Piálek, J., and N. H. Barton. 1997. The spread of an advantageous allele across a barrier: the effects of random drift and selection against heterozygotes. Genetics 145:493–504.

Pocock, M. J. O., J. B. Searle, and P. C. L. White. 2004. Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. J. Anim. Ecol. 73:878–888.

Pocock, M. J. O., H. C. Hauffe, and J. B. Searle. 2005. Dispersal in house mice. Biol. J. Linn. Soc. 84:565–583.

Polechová, J., and N. Barton. 2011. Drift widens the expected cline but narrows the expected cline width. Genetics, submitted.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences and solutions. Nat. Rev. Genet. 6:847–859.

Porter, A. H., R. Wenger, H. Geiger, A. Scholl, and A. M. Shapiro. 1997. The *Pontia daplidice-edusa* hybrid zone in northwestern Italy. Evolution 51:1561–1573.

Prager, E. M., P. Boursot, and R. D. Sage. 1997. New assays for Y chromosome and p53 pseudogene clines among East Holstein house mice. Mamm. Genome 8:279–281.

Prager, E. M., R. D. Sage, U. Gyllensten, W. K. Thomas, R. Hübner, C. S. Jones, L. Noble, J. B. Searle, and A. C. Wilson. 1993. Mitochondrial DNA sequence diversity and the colonization of Scandinavia by house mice from East Holstein. Biol. J. Linn. Soc. 50:85–122.

Presgraves, D. C. 2008. Sex chromosomes and speciation in *Drosophila*. Trends Genet. 24:336–343.

Qvarnström, A., and R. I. Bailey. 2009. Speciation through evolution of sex-linked genes. Heredity 102:4–15.

Raufaste, N., A. Orth, K. Belkhir, D. Senet, C. Smadja, S. J. E. Baird, F. Bonhomme, B. Dod, and P. Boursot. 2005. Inferences of selection and migration in the Danish house mouse hybrid zone. Biol. J. Linn. Soc. 84:593–616.

Rieseberg, L. H., and J. H. Willis. 2007. Plant speciation. Science 317:910–914.

Rieseberg, L. H., J. Whitton, and K. Gardner. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics 152:713–727.

Riginos, C., and C. W. Cunningham. 2005. Local adaptation and species segregation in two mussel (*Mytilus edulis* x *Mytilus trossulus*) hybrid zones. Mol. Ecol. 14:381–400.

Sætre, G. P., T. Borge, K. Lindroos, J. Haavie, B. C. Sheldon, C. Primmer, and A. C. Syvanen. 2003. Sex chromosome evolution and speciation in *Ficedula* flycatchers. Proc. R. Soc. Lond. B 270:53–59.

Sage, R. D. 1981. Wild mice. Pp. 39–90 *in* H. L. Foster, J. D. Small, and J. G. Fox, eds. The mouse in biomedical research. Vol. 1. Academic Press, New York.

Sage, R. D., J. B. Whitney, and A. C. Wilson. 1986. Genetic analysis of a hybrid zone between *domesticus* and *musculus* mice (*Mus musculus* complex): Hemoglobin polymorphism. Curr. Top. Microbiol. Immunol. 127:75–85.

Sage, R. D., W. R. Atchley, and E. Capanna. 1993. House mice as models in systematic biology. Syst. Biol. 42:523–561.

Shifman, S., J. T. Bell, R. R. Copley, M. S. Taylor, R. W. Williams, R. Mott, and J. Flint. 2006. A high-resolution single nucleotide polymorphism genetic map of the mouse genome. PLoS Biol. 4(12):e395.

Sites, J. W., N. H. Barton, and K. M. Reed. 1995. The genetic structure of a hybrid zone between two chromosome races of the *Sceloporus grammicus* complex (Sauria, Phrynosomatidae) in central Mexico. Evolution 49:9–36.

Slatkin, M. 1973. Gene flow and selection in a cline. Genetics 75:733–756.

Slatkin, M., and T. Maruyama. 1975. Genetic drift in a cline. Genetics 81:209–222.

Storchová, R., S. Gregorová, D. Buckiová, D. Kyselová, P. Divina, and J. Forejt. 2004. Genetic analysis of X-linked hybrid sterility in the house mouse. Mamm. Genome 15:515–524.

Stuckas, H., K. Stoof, H. Quesada, and R. Tiedemann. 2009. Evolutionary implications of discordant clines across the Baltic *Mytilus* hybrid zone (*Mytilus edulis* and *Mytilus trossulus*). Heredity 103:146–156.

Swofford, D. L., G. J. Olsen, P. J. Waddel, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. 2nd ed. Sinauer Associates, Inc., Sunderland, MA.

Szymura, J. M., and N. H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40:1141–1159.

———. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45:237–261.

Teeter, C. K., B. A. Payseur, L. W. Harris, M. A. Bakewell, L. M. Thibodeau, J. E. O′Brien, J. G. Krenz, M. A. Sans-Fuentes, M. W. Nachman, and P. K. Tucker. 2008. Genome-wide patterns of gene flow across a house mouse hybrid zone. Genome Res. 18:67–76.

Teeter, C. K., L. M. Thibodeau, Z. Gompert, C. A. Buerkle, M. W. Nachman, and P. K. Tucker. 2010. The variable genomic architecture of isolation between hybridizing species of house mice. Evolution 64:472–485.

Tucker, P. K., R. D. Sage, J. Warner, A. C. Wilson, and E. M. Eicher. 1992. Abrupt cline for sex chromosomes in a hybrid zone between two species of mice. Evolution 46:1146–1163.

Vanlerberghe, F., B. Dod, P. Boursot, M. Bellis, and F. Bonhomme. 1986. Absence of Y-chromosome introgression across the hybrid zone between *Mus musculus domesticus* and *Mus musculus musculus*. Genet. Res. 48:191–197.

Vanlerberghe, F., P. Boursot, J. Catalan, S. Gerasimov, F. Bonhomme, B. A. Botev, and L. Thaler. 1988a. Analyse génétique de la zone d′hybridation entre les deux sous-espéces de souris *Mus musculus domesticus* et *Mus musculus musculus* en Bulgarie. Genome 30:427–437.

Vanlerberghe, F., P. Boursot, J. T. Nielsen, and F. Bonhomme. 1988b. A steep cline for mitochondrial DNA in Danish mice. Genet. Res. 52:185–193.

Vines, T. H., S. C. Köhler, A. Thiel, I. Ghira, T. R. Sands, C. J. MacCallum, N. H. Barton, and B. Nürnberger. 2003. The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*. Evolution 57:1876–1888.

Widmer, A., C. Lexer, and S. Cozzolino. 2009. Evolution of reproductive isolation in plants. Heredity 102:31–38.

Wright, S. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. Univ. Chicago Press, Chicago.

Yanchukov, A., S. Hofman, J. M. Szymura, S. V. Mezhzherin, S. Y. Morozov-Leonov, N. H. Barton, and B. Nürberger. 2006. Hybridization of *Bombina bombina* and *B. variegata* (Anura, Discoglossidae) at a sharp ecotone in western Ukraine: Comparisons across transects and over time. Evolution 60:583–600.

Yang, H., Y. Ding, L. M. Hutchins, J. Szatkiewicz, T. A. Bell, B. J. Paigen, J. H. Graber, F. Pardo-Manuel de Villena, and G. A. Churchill. 2009. A customized and versatile high-density array for the mouse. Nat. Methods 6:663–666.

Associate Editor: N. Barton

Appendix 1. **List of localities studied in the Czech transects ordered by geographic distance from the *M. m. domesticus* side to the *M. m. musculus* side. For each locality, country of origin (D=Bavaria, Germany; CZ=Czech Republic), distance along the transect in kilometers (Dist), and the number of chromosomes scored (NCh) are given. The numbers of localities correspond to Figure 1B. For allele frequencies at the 13 X-linked loci see Table S2. Similar information on localities in the Bavarian transect is listed in Table 1 of Payseur et al. (2004).**

No.	Locality	Dist	NCh	No.	Locality	Dist	NCh
1.	Kübelhof 1, D	0.00	4	34.	Dolnice, CZ	67.11	32
2.	Weidesgrün 1, D	15.63	11	35.	Doubí 3, CZ	67.29	34
3.	Straas 2, D	22.54	29	36.	Mlýnek 1, CZ	67.41	2
4.	Straas 1, D	22.73	20	37.	Jindřichov, CZ	67.82	24
5.	Münchberg, D	24.20	3	38.	Milhostov 1, CZ	68.64	32
6.	Benk, D	29.30	36	39.	Nebanice 3, CZ	72.01	19
7.	Lehsten, D	35.29	38	40.	Nebanice 2, CZ	72.24	12
8.	Röslau, D	41.64	3	41.	Krajková 2, CZ	72.30	10
9.	Hebanz, D	43.59	13	42.	Dolina, CZ	72.51	20
10.	Plössberg, D	45.63	8	43.	Krajková 3, CZ	72.64	13
11.	Unterweißenbach, D	46.01	8	44.	Kaceřov 1, CZ	72.77	25
12.	Thierstein, D	48.52	25	45.	Kaceřov 2, CZ	72.89	54
13.	Höchstädt-Zelch, D	49.08	5	46.	Obilná 1, CZ	72.93	55
14.	Neuenreuth, D	50.59	43	47.	Mostov, CZ	73.34	58
15.	Neuenreuth 8, D	50.97	34	48.	Anenská Ves, CZ	73.42	2
16.	Polná, CZ	55.55	5	49.	Chotíkov 1, CZ	73.89	18
17.	Libá 2, CZ	55.89	17	50.	Boučí, CZ	74.39	13
18.	Libá 1, CZ	56.00	17	51.	Hlavno, CZ	78.82	4
19.	Hazlov, CZ	57.16	13	52.	Rudolec 1, CZ	80.75	16
20.	Hůrka 1, CZ	57.77	62	53.	Rudolec 2, CZ	80.97	43
21.	Poustka 1, CZ	58.63	13	54.	Kostelní Bříza 1, CZ	81.91	17
22.	Plesná, CZ	58.91	2	55.	Staré Sedlo, CZ	85.31	17
23.	Poustka 2, CZ	59.67	27	56.	Horní Slavkov, CZ	91.74	33
24.	Lužná, CZ	60.84	61	57.	Nová Ves okál, CZ	93.23	9
25.	Starý Rybník, CZ	62.29	13	58.	Nová Ves porod., CZ	93.29	35
26.	Křižovatka, CZ	63.12	4	59.	Teleč, CZ	109.68	3
27.	Nový Kostel, CZ	64.19	11	60.	Těšetice, CZ	110.37	4
28.	Horní Ves, CZ	65.21	19	61.	Týnistě 2, CZ	117.26	19
29.	Dolní Pelhřimov, CZ	65.61	6	62.	Podboř. Rohozec, CZ	118.71	7
30.	Dlouhé Mosty, CZ	65.72	64	63.	Vrbička, CZ	123.32	9
31.	Nový Drahov, CZ	65.78	29	64.	Buškovice 1, CZ	126.58	10
32.	Nová Ves, CZ	65.78	19	65.	Buškovice 2, CZ	126.58	22
33.	Svatý Kříž, CZ	67.04	5	66.	Buškovice 5, CZ	126.58	3

Supporting Information

The following supporting information is available for this article:

Table S1. Individual genotypes at 13 X-linked SNPs at localities across the Czech house mouse hybrid zone (Excel file).
Table S2. Allele frequencies of 13 SNPs across the Czech hybrid zone (Excel file).
Table S3. Maximum-likelihood estimates of cline parameters based on the BMF and AMF search scenarios for the Czech and Bavarian transects (Excel file).
Figure S1. Distribution of *M. m. musculus* allele frequencies (ordinate) plotted against the distance in kilometers along the transect (abscissa) for 13 X-linked loci in the Czech (left panels) and Bavarian (right panels) transects.
Figure S2. Jackknife values obtained for 13 X-linked loci in the Czech (left panels) and Bavarian (right panels) transects using BMF. Cline widths (abscissa) are plotted against corresponding maximum likelihood values (ordinate). Estimates resulting from the full dataset (66 and 31 localities from the Czech and Bavarian transect, respectively) are also shown for the BMF (red symbol), AMF1 (green symbol), and AMF100 (blue symbol) scenarios; see text for explanation of the searching scenarios.

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