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The genus Mus as a model for evolutionary studies

Edited by J. Britton-Davidian and J. B. Searle

Testing for selection on the androgen-binding protein in the Danish mouse hybrid zone

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The three peripheral subspecies of the house mouse *Mus musculus* have fixed specific variants of the androgen-binding protein (ABP) that have been proposed to be part of a recognition mechanism that could participate in sexual isolation between the subspecies. We tested for selection on *Abpa* by characterizing the pattern of *Abpa* introgression across a transect of the hybrid zone between *M. m. musculus* and *M. m. domesticus* in Jutland. On the *musculus* side, the cline for *Abpa* resembled that of a nearly neutral allozyme more than that of strongly selected X and Y chromosome markers. However, the high central step which displaces the tail of introgression of *Abpa* to higher frequencies was best accounted for by linkage to a locus under strong selection against hybrids. Still, we cannot exclude that this pattern results from weak selection against *Abpa* in the tail of introgression, which would be compatible with an assortative choice mechanism. On the *domesticus* side there was little introgression close to the hybrid zone, presumably due to a geographical barrier to migration. However, substantial frequencies of *musculus* alleles occurred further away, suggesting mixed colonization patterns as well as flow across the hybrid zone. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, 84, 447–459.

ADDITIONAL KEYWORDS: assortative mating – gene flow – genetic barrier – *Mus musculus domesticus* – *Mus musculus musculus*.

INTRODUCTION

Androgen-binding protein (ABP) is a member of the secretoglobulin family (Laukaitis & Karn, 2005, this issue) and a major component of saliva that mice transfer to their pelts and territories when grooming. The role this protein plays is unknown, but, as it is able to bind male sex steroid hormones and because distinct structural variants have been fixed alternatively in the peripheral subspecies of the house mouse *Mus musculus*, it has been proposed to be part of a mate recognition mechanism (Karn & Dlouhy, 1991). This idea was reinforced considerably by the results of

behavioural studies (Laukaitis, Crister & Karn, 1997) and odour preference tests (Talley, Laukaitis & Karn, 2001), which showed that female mice belonging to inbred and outbred strains of mice derived from *M. m. musculus* and *M. m. domesticus* made assortative mate choices based on their ABP genotypes.

The rapid evolution of the gene coding for the alpha subunit of this dimeric protein (*Abpa*) is also consistent with a role in mediating assortative mating. The alleles found in the peripheral subspecies show a large excess of non-synonymous differences and a lack of both exon and intron polymorphism (Hwang *et al.*, 1997; Karn & Nachman, 1999; Karn *et al.*, 2002). This pattern of evolution suggests that the new ABP variants were fixed in a series of selective sweeps. How-

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ever, the situation prevailing in the central parts of the species range does not support this idea (Karn *et al.*, 2002). Not only is there extensive intron and exon polymorphism, but there is also evidence for secondary admixture of subspecies-specific *Abpa* variants in the regions of transition between the peripheral subspecies and the central populations. Furthermore, these authors also found phylogenetic evidence that interspecific exchanges between *M. musculus* and the three other Palaearctic species, *M. spretus*, *M. spicilegus* and *M. macedonicus*, may have occurred in the past.

Our aim was to investigate the role that selection plays in shaping the introgression of *Abpa* in the hybrid zone that results from the secondary contact between *M. m. domesticus* and *M. m. musculus* in Europe (for review see Boursot *et al.*, 1993). Early studies on three different transects of this zone in Denmark, Germany and Bulgaria showed them all to be characterized by narrow genetic transitions of similar widths (Hunt & Selander, 1973; Sage, Whitney & Wilson, 1986; Vanlerberghe *et al.*, 1988a; Vanlerberghe *et al.*, 1988b; Prager *et al.*, 1993). In the Danish and Bavarian transects, the clines of both sex chromosome markers are much narrower compared with those of the autosomal allozymes (Tucker *et al.*, 1992; Dod *et al.*, 1993), which suggests that genes on both sex chromosomes are more strongly affected by hybrid dysgenesis than are the autosomal loci. The limited introgression of the Y chromosome in Bulgaria (Vanlerberghe *et al.*, 1986) and East Holstein (Prager, Boursot & Sage, 1997) also supports this idea. However, a preliminary study across a transect in Western Bohemia (Munclinger *et al.*, 2002) suggests that the situation is more complex than originally supposed, as here the centres of the clines for the two sex chromosomes are not concordant and the Y transition appears to occur over a wide area. Raufaste *et al.* (2005, this issue) showed that eight allozymes on six different autosomal chromosomes could be characterized by a series of concordant clines with central steps in frequency flanked by smooth tails of introgression. This is consistent with the idea that this hybrid zone is a tension zone maintained by the balance between migration and selection against hybrids (Barton & Hewitt, 1985). The shapes of the allozyme clines suggest that the loci themselves are only weakly selected against but are submitted to an overall barrier to gene flow created by selection against other loci in hybrid genomes.

To test for selection on *Abpa* we compared its introgression pattern across the Danish transect of the hybrid zone in Jutland with those of an X and a Y chromosome marker that are both strongly affected by selection against hybrids, as well as with those of the weakly selected allozymes mentioned above.

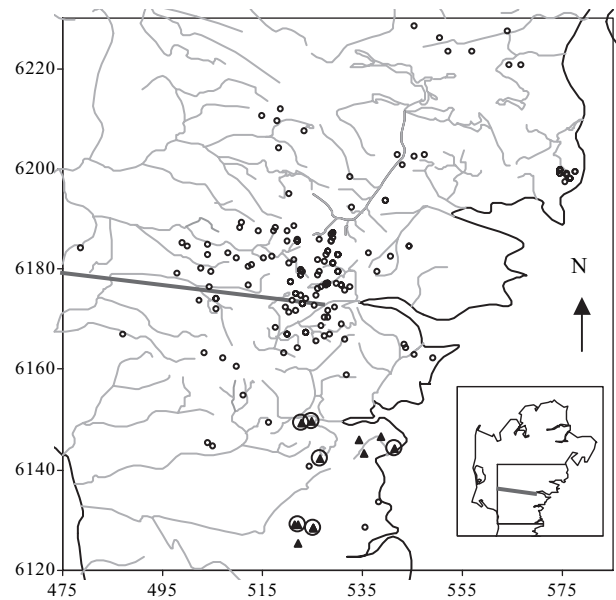


Figure 1. Map showing the geographical position of the *Mus musculus musculus* samples on the transect and the centre of the hybrid zone. Latitude and longitude are given as universal transverse mercator (UTM) grid coordinates (in km). The estimated centre of the transect is shown as a thick grey line. The position of the transect is indicated on the map of the Jutland peninsula in the insert. The filled triangles on the southernmost part of the map correspond to the localities with the least overall introgression referred to in the text. Those that are introgressed for *Abpa* are encircled.

METHODS

MICE

Mice were live-trapped inside buildings using multi-capture traps along a transect of the hybrid zone in Jutland, during seven field trips between 1984 and 2000. The localities sampled are shown in Figure 1. A number of localities were sampled more than once and were treated as independent samples. The list of samples with their geographical position in universal transverse mercator (UTM) grid coordinates is given in the Appendix.

GENETIC ANALYSIS

DNA from spleen or tail tissue was extracted using standard phenol/chloroform procedures. An allele-specific PCR reaction was used to type the *Abpa* variants. The two forward primers included the fixed non-synonymous differences in the exon sequences of *Abpa^a* (*M. m. domesticus*) and *Abpa^b* (*M. m. musculus*) while the reverse primers fell in the following intronic sequence and were not subspecies specific (Karn *et al.*, 2002). Two PCR reactions were performed in 10- μ L

aliquots in the presence of 25 ng DNA and 0.125 U Taq polymerase with 2- and 2.5-mM MgCl₂ for the *Abpa*^a and *Abpa*^b primer pairs, respectively. After an initial incubation at 95 °C for 3 min the reactions were amplified for 35 cycles. The annealing and elongation conditions are given with the primer sequences in Table 1. The two amplification products were analysed on the same 1.5% agarose gels. The shorter one (*Abpa*^a, 192 bp) was loaded first and run for 15 min before the longer one (*Abpa*^b, 290 bp) was loaded.

The *Zfy2* deletion in the *M. m. musculus* Y chromosome (Boissinot & Boursot, 1997) and a SINE (B1) insertion in the *Btk* gene in the *M. m. domesticus* X chromosome (Munclinger, Boursot & Dod, 2003) were used to differentiate between the two subspecies.

The eight allozyme loci used to calculate the average autosomal locus referred to in this study were: *Amy1*, chromosome (chr.) 3; *Es1*, *Es2*, chr. 8; *Es10*, chr. 14; *Gpd*, chr. 4; *Mpi*, chr. 9; *Np*, chr. 14 and *Pgm*, chr. 5. None of these markers are on the same chromosome as *Abpa*, which is 10 cM from the centromere of chromosome 7.

CLINE ANALYSIS

The variations of allele frequencies were studied along the direction of the maximum frequency gradient, which was determined using the eight allozyme loci, as described in detail in Raufaste *et al.* (2005, this issue). The shapes of the clines describing the change in allele frequencies of the different loci along a one-dimensional representation of the transect were determined by comparing the maximum likelihood estimations of three single-locus cline models of increasing complexity using the computer package ANALYSE (by N. Barton and S. Baird, <http://helios.bto.ed.ac.uk/evolgen/Mac/Analyse/>). The simplest was a symmetrical sigmoidal model in which only the geographical position of the centre and width were varied. The next was a symmetrical cline model with a central step and two symmetrical tails of introgression, described by two additional parameters. The most complex model was asymmetrical with two further parameters that allowed for different tails of introgression on either side. Likelihood ratio tests

were then used to choose the best acceptable model with a minimum number of parameters. The parameters describing the most complex model were the position of the cline centre, cline width (w), which is the inverse of the maximum frequency gradient in the central step, $B0/w$ and $B1/w$, the ratios of the barrier to gene flow to the width of the clines on the left and right hand sides of the centre, respectively, and θ_0 and θ_1 , which correspond to the square of the rate of decay of allele frequencies in the tails of introgression. B represents the barrier to gene flow and is the distance of habitat that would have to be crossed by a neutral allele in order to observe a change of frequency equivalent to that caused by the central step. The value of θ can be used to estimate the ratio of the selection on the locus itself to the overall selection (Barton & Hewitt, 1985; Barton & Gale, 1993). A detailed description of the procedures used to find the direction of maximum gradient, to correct sample sizes for inbreeding and random fluctuations, and to fit the cline shapes is given in Raufaste *et al.* (2005, this issue). For the haplo/diploid X chromosome locus the males and females were first analysed separately and, as no significant difference between the sexes was found, they were then grouped.

RESULTS

The *musculus* allele frequencies of the *Abpa*, *Zfy* (on the Y chromosome) and *Btk* (on the X chromosome) loci were measured in a large number of samples along a transect of the hybrid zone between *M. m. musculus* and *M. m. domesticus* in Jutland. The *musculus* allele frequencies of the three loci in the different samples are given in the Appendix together with the effective sample size, the name of the locality, the sampling year, its geographical position and the derived coordinate that defined its position on the one-dimensional cline representation. The average *musculus* allele frequency of the eight allozymes taken from Raufaste *et al.* (2005, this issue) are also included.

The variations of the *musculus* allele frequencies of *Abpa*, *Zfy*, *Btk* and the average frequency of eight autosomal loci across the one-dimensional transect are shown in Figure 2. The Y chromosome behaved

Table 1. Primer sequences and the annealing and elongation conditions used to amplify the *Abpa* fragments specific to *Mus musculus domesticus* (*Abpa*^a) and *M. m. musculus* (*Abpa*^b)

Primer pair	Forward	Reverse	Annealing		Elongation	
			°C	s	°C	s
<i>Abpa</i> ^a	GAAACAATTCAATGAAAACACTAAAG	TGTGCCACTGCTCTGTATTC	55	30	72	30
<i>Abpa</i> ^b	ACAATTCAATGAAAACCGTGA	AAACTTGGGCAGGGATTAG	60	90	72	90

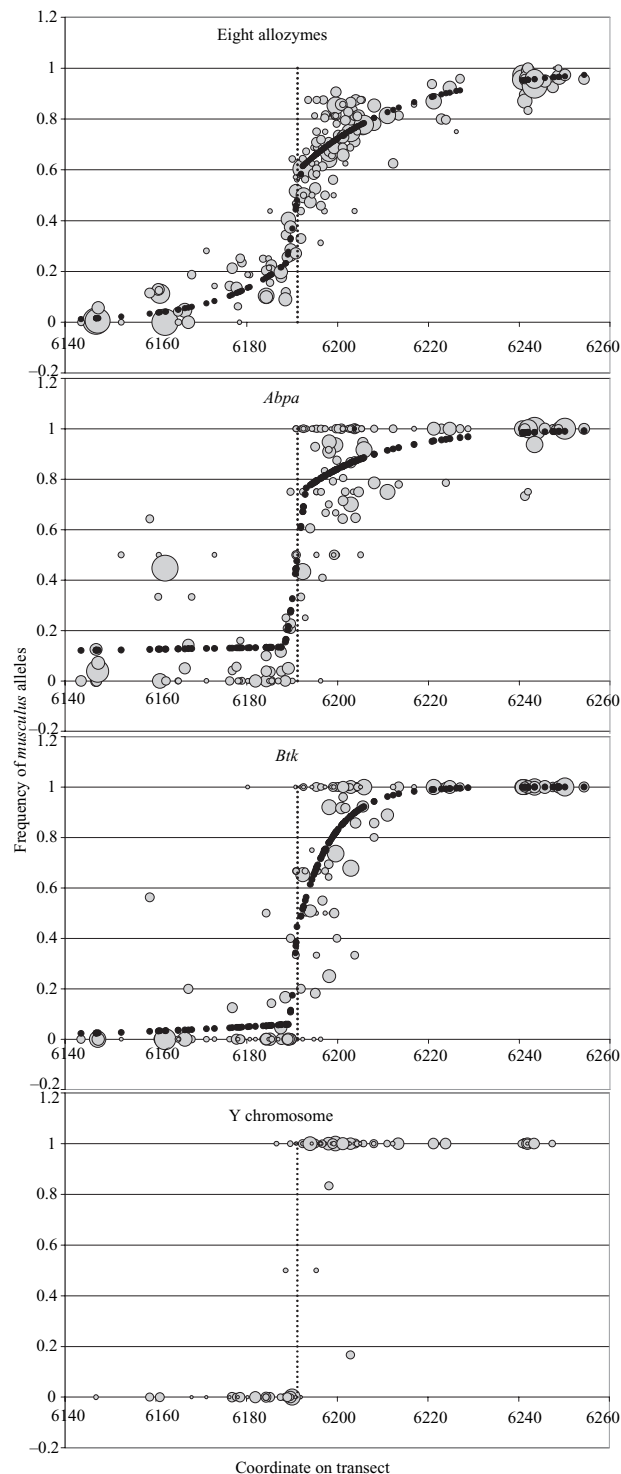


Figure 2. Variations of *Mus musculus musculus* allele frequencies along the one-dimensional transect across the hybrid zone. The grey spots correspond to the real data and their area is proportional to the effective sample size. The black spots give the best cline fit and the position of the centre of the transect is represented by the vertical dotted line.

very differently compared with the other three loci. The transition from the *domesticus* to the *musculus* variant was almost complete within a few km from the centre of the hybrid zone. The Y chromosome is therefore clearly under very strong selection in the hybrid genome.

The shapes of the clines for the *Abpa* and *Btk* loci were fitted to the three classes of models and it can be seen in Table 2 that in both cases the likelihood ratio test showed that the most complex model, corresponding to a stepped cline with asymmetrical tails of introgression, was significantly better. The maximum likelihood positions of the centre of both these loci coincided with that determined for the eight allozymes by Raufaste *et al.* (2005, this issue). Although the cline models used for these two loci were not applicable to the very abrupt Y transition it is obvious that it occurred in much the same place as the other loci. The shapes of the fitted clines are shown in Figure 2.

On the *domesticus* side of the transect, the introgression of *Btk* was limited to a small number of localities (7 of 52) spread over a wide geographical area. On the other side of the zone, an extremely variable range of *musculus* allele frequencies (from 0.2 to 1) was found in the first 16 km, but then they increased rapidly so that all the populations further than 20 km from the centre were fixed. This was modelled by a steep frequency step of almost 0–0.5 on the *domesticus* side followed by a rapid exponential decay on the *musculus* side. In fact, most of the admixture occurred on the *musculus* side of the inferred centre. Although this atypical cline shape made the estimated selection parameters unreliable, the *Btk* locus clearly showed much less introgression compared with the average autosomal locus, and is obviously under much stronger selection.

The frequency changes of the *Abpa* alleles did not follow the same pattern as those of *Btk*. Although there was also a sharp step on the *domesticus* side, it only continued for about 2 km beyond the centre. This means that, although most of the intermixing also occurred on the *musculus* side, it spanned a much narrower spectrum of frequencies (mostly above 0.6) than did *Btk*. Furthermore, this zone of admixture stretched out as far as 58 km from the centre. As a result the fitted cline had a high central frequency step with a rather steep gradient that was flanked by a right hand tail which decayed slowly but was clearly displaced to higher *musculus* frequencies compared with the average allozyme cline. The parameters defining the best cline fits for these two loci (Table 2) suggest that there is a stronger barrier to the introgression of *Abpa* ($B1 = 50$ km) than there is for the average autosomal locus ($B1 = 20$ km), even though both had similar rates of decay ($\theta_1 = 0.029$ and 0.033 , respectively). Table 3 compares the cline parameters

Table 2. Test of different cline models for the *Abpa* and *Btk* loci, and comparison with the best fit for the eight allozyme loci

Locus/model	<i>w</i> (km)	Centre	<i>B0/w</i>	θ_0	<i>B1/w</i>	θ_1	LL	2DLL	d.f.	<i>P</i>
<i>Abpa</i>										
Sigmoid (2 parameters)	48.2	6188.3					-129.89			
Stepped symmetric (4 parameters)	7.3	6191.4	18.9	0.009			-93.50	72.77	2	2×10^{-16}
Stepped asymmetric (6 parameters)	6.3	6191.2	3590.4	4×10^{-7}	7.1	0.029	-83.83	19.34	2	6×10^{-5}
<i>Btk</i>										
Sigmoid (2 parameters)	19.8	6193.5					-103.83			
Stepped symmetric (4 parameters)	12.9	6194.1	4.6	0.198			-90.73	26.20	2	2×10^{-6}
Stepped asymmetric (6 parameters)	3.0	6191.2	97.0	0.001	1.6	0.041	-81.41	18.63	2	9×10^{-5}
Eight allozymes										
Stepped asymmetric (6 parameters)	8.9	6191.2	2.2	0.072	2.2	0.033				

LL, log-likelihood; 2 DLL, twice the difference between the LLs of two models.

Table 3. Comparison of the parameters of the clines on the *musculus* side of the hybrid zone, for eight allozymes and *Abpa*

Locus	<i>w</i> (km)	(2LL limits)	<i>B1</i>	(2LL limits)	θ_1	(2LL limits)
<i>Amy1</i>	20.6	(15.2–26.4)	199.0	(64.4–2148.0)	0.037	(0.001–0.157)
<i>Es1</i>	11.0	(2.2–25.2)	7.2	(0.6–25.6)	0.172	(0.010–0.999)
<i>Es10</i>	6.4	(4.9–11.7)	25.3	(13.9–58.9)	0.016	(0.008–0.055)
<i>Es2</i>	3.8	(1.3–9.2)	24.2	(10.1–53.6)	0.003	(0.000–0.019)
<i>Gpd</i>	6.3	(3.4–13.0)	6.9	(4.2–16.8)	0.032	(0.009–0.135)
<i>Mpi</i>	6.2	(2.2–18.9)	18.7	(4.6–52.4)	0.041	(0.004–0.453)
<i>Np</i>	5.9	(3.8–44.6)	14.0	(5.9–137.3)	0.009	(0.002–0.715)
<i>Pgm</i>	6.8	(4.0–9.1)	16.3	(12.7–30.2)	0.035	(0.010–0.061)
Eight loci	8.9	(7.7–12.4)	19.9	(13.7–28.1)	0.033	(0.023–0.063)
<i>Abpa</i>	6.3	(3.7–10.2)	44.5	(16.0–103.7)	0.029	(0.009–0.104)

2LL limits, two log-likelihood support limits.

obtained for the eight allozyme loci individually (taken from Raufaste *et al.*, 2005, this issue), the average allozyme locus and *Abpa* on the *musculus* side of the transect. Although there were variations across loci, the width of the *Abpa* cline was comparable to those of most of the allozyme loci, but its barrier tended to be higher. The only locus with a higher barrier was *Amy* which had a much wider atypical cline. The rate of decay θ_1 for *Abpa* fell within the range of values found for the other loci. However, one should note that the two log-likelihood limits of these parameter estimates were quite wide, and most intervals overlapped across loci.

The situation for *Abpa* on the *domesticus* side was a bit more confusing. Although *musculus* alleles appeared to decay rapidly immediately south of the centre, they occurred again, sometimes at rather high frequencies, in a large number of populations further south. As a result, the best fit was a flat tail of introgression that provided a very poor description of the data. *Btk* also showed a rather flat decay on the

domesticus side and showed occasional sporadic introgression, like several of the allozymes studied by Raufaste *et al.* (2005, this issue). As a consequence, the cline parameters fitted on the *domesticus* side did not appear to be very meaningful, and the model applied did not provide a satisfactory representation of the data. The possible reasons for this are discussed in more detail in Raufaste *et al.* (2005, this issue). We therefore did not attempt to compare the left hand introgression tail parameters across loci but rather examined the levels of sporadic introgression that occurred at several of the loci. The distribution of the *musculus* allele frequencies of *Abpa* in the samples from the localities south of the Kolding Fjord more than 20 km from the transect centre (the first 19 samples in the Appendix), differed significantly from that of the average autosomal locus (Wilcoxon matched pairs test, $P < 0.047$). To investigate the reasons for this difference we compared the frequency distribution of *Abpa musculus* alleles with those of *Amy1*, *Es1*, *Es10*, *Mpi*, *Np* and *Pgm*. Data were missing for *Es2*

and *Gpd* in six and nine of these samples, respectively, so they could not be included in the tests. A Friedman two-way analysis of variance of the *musculus* allele frequencies of the six allozyme markers in 16 samples (samples 187, 189 and 92 were not considered because of missing data) showed that only three of them were introgressed for *musculus* alleles to a significantly different extent (with all 16 localities as variables, $P < 0.00015$, and when the samples with the three highest ranks, 87, 88 and 174, were removed, $P < 0.06382$). A second Friedman test was done on the 13 samples with the lowest degree of allozyme introgression with *Abpa* and the six allozyme loci taken as variables (with all seven loci, $P < 0.00127$, with *Abpa* removed, $P < 0.01648$, and with *Amy1* removed, $P < 0.25057$). These results showed the *Abpa* and *Amy1* *musculus* alleles to be significantly more widespread compared with those of *Es1*, *Es10*, *Mpi*, *Np* and *Pgm* in this region. The positions of these samples are indicated as triangles on the map in Figure 1 with the localities introgressed for *Abpa* encircled and it can be seen that they are not all clustered in one particular geographical area.

DISCUSSION

Two earlier studies on rather limited datasets suggested that the sex chromosomes are under stronger selection in the hybrid genome than is the average autosomal locus (Tucker *et al.*, 1992; Dod *et al.*, 1993). Our results on a much larger sample confirm that both the Y chromosome and the X chromosome locus *Btk* are under strong selection in the Jutland transect. We have also shown that the Y chromosome transition in the centre of the hybrid zone is even more abrupt than our preliminary study suggested (Dod *et al.*, 1993). Very few localities harbour both variants or a Y variant that does not correspond to the overall local genetic background. It should be noted that the sample with a high frequency of *domesticus* variants on the *musculus* side of the zone, more than 10 km from the centre, was from Givskud zoo. The introgression found is almost certainly due to escapees from their mouse-breeding colony which was closed soon after this sample was caught (1991). None of the males caught in 1987 and 1998 in the same place carried a *domesticus* Y.

Assessing the involvement of selection in shaping the *Abpa* cline is not straightforward. We have seen that the central step of the *Abpa* cline is higher compared with that of the average autosomal locus, which means that the right hand tail of introgression is displaced to higher *musculus* frequencies. The most likely estimate of the cline parameter *B1*, given in Table 3, suggests that *Abpa* is subjected to a stronger barrier to gene flow than are most allozymes. How-

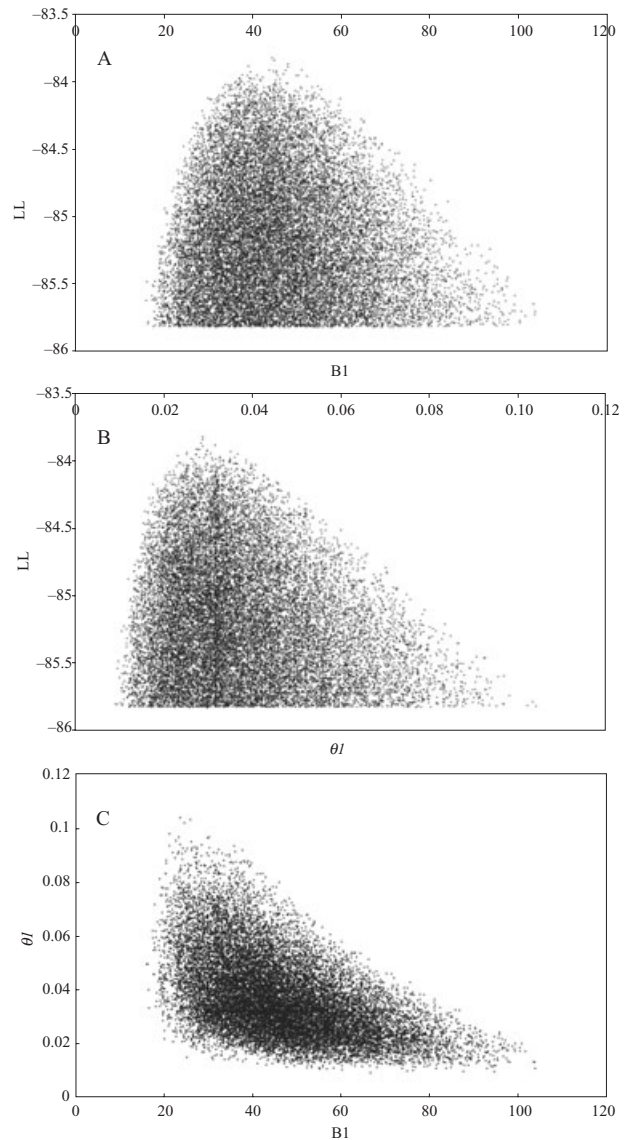


Figure 3. Likelihood (LL) surface of the values of *B1* (A) and θ_1 (B) that fall within the two log-likelihood support limits. (C) envelope of the corresponding values of θ_1 and *B1* for all the models that fall within the confidence interval.

ever, the corresponding estimate of the parameter θ_1 , which reflects the ratio of the selection on the locus itself to the overall selection caused by the rest of the genome, suggests that *Abpa* itself is not submitted to significantly stronger selection than are the allozymes. This situation could result from the linkage of *Abpa* to a locus under strong selection in the centre of the hybrid zone. However, we have seen that there is considerable uncertainty with respect to these parameter estimates. Figure 3 gives graphical representations of the log-likelihood support limits for *B1* and θ_1 for the *Abpa* cline and it can be seen that they are negatively correlated so that the *Abpa* data could also be

described acceptably by a barrier as low as that found for the average allozyme locus (around 20 km) but with a higher value for the parameter θ_i . If this were the case it would imply direct selection on *Abpa* in the tail of introgression. Assuming a barrier for *Abpa* of the order of 20 km, the maximum acceptable value for parameter θ_i would be around 0.1 (Fig. 3), which implies direct selection on *Abpa* of the order of 0.005 if the intensity of the overall selection acting in the hybrid zone is taken as 0.05, as estimated by Raufaste *et al.* (2005, this issue). However, it is not certain that such a small selection coefficient can be measured with any confidence using this kind of approach.

The situation on the *domesticus* side of the zone cannot be interpreted in the same way. The asymmetry of the *Abpa* cline is due to a very abrupt frequency change on the *domesticus* side of the estimated centre. This is also the case for a number of the allozyme markers and Raufaste *et al.* (2005, this issue) suggested that it is at least partially due to the influence of a geographical barrier to gene flow that occurs on the *domesticus* side of the zone. Such a barrier would contribute to the abruptness of the frequency change observed for *Abpa* but it makes the high frequency of *musculus* *Abpa* alleles found further south even more surprising. Recent long-distance migration from the *musculus* side of the transect is unlikely to explain all the observed *Abpa* introgression since it is not geographically aggregated and in the majority of the localities concerned the only other locus introgressed to a similar extent is *Amy1*. A possible explanation is that the *domesticus* mice which colonized south Jutland were already introgressed for certain loci before they arrived. All the islands along the eastern side of the Jutland peninsula have *musculus* populations and secondary contact between the two subspecies occurs further south in Holstein (Prager *et al.*, 1993) and in northern Germany, so opportunities for introgression were multiple. It has been shown that the takeover of *M. m. musculus* mtDNA by a *domesticus* haplotype in Northern Jutland (Ferris *et al.*, 1983) occurred at the time of colonization, rather than by flow across the hybrid zone (Vanlerberghe *et al.*, 1988b). The settlement of farmers within the last 150 years in large areas of previously unoccupied land in Jutland could also have contributed to sporadic introgression. Whatever their origin, the persistence of such high frequencies of *musculus* *Abpa* alleles makes it unlikely that this variant is selected against in a *domesticus* background.

In conclusion, we find that *Abpa* seems to be more affected by selection in the hybrid zone than is the average autosomal locus. The most likely explanation is linkage to a gene under selection in the hybrid genome. However, we cannot exclude the possibility that the pattern is due to weak selection on *Abpa* itself in the *musculus* tail of introgression,

which would be consistent with the behavioural results of Bímová, Karn & Piálek (2005, this issue), who found some evidence for weak preferences at the edges of a Czech transect of the hybrid zone. However, before considering the possibility that behaviour at the edges of the cline influences the shape of the introgression tails, as suggested earlier by Laukaitis *et al.* (1997), we need better estimates of selection than we were able to make in the complex Jutland transect.

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APPENDIX

Locality, sampling year, geographical coordinates, derived coordinate that defines its position on the one dimensional cline, frequency of *Mus musculus musculus* alleles (fr_{mus}) and effective sample size (N_e) of each sample for loci *Abpa*, *Btk*, *Zfy* and the average autosomal locus. Numbers in the first column refer to the samples analysed in Rautaste *et al.* (2005, this issue).

No.	Locality	Year	Longitude	Latitude	Transect	<i>Btk</i>		Average autosomal locus		<i>Abpa</i>		<i>Zfy</i>	
						<i>fr mus</i>	N_e	<i>fr mus</i>	N_e	<i>fr mus</i>	N_e	<i>fr mus</i>	N
186	Ringtved	2000	522.13	6125.52	6143.49	0.00	4.00	0.00	14.00	0.00	6.00		
115	Simmersted A	1985	525.00	6128.50	6146.80	0.00	6.27	0.00	54.00	0.00	6.76	0.00	4.00
114	Simmersted	1991	525.00	6128.50	6146.80	0.00	4.92	0.00	195.00	0.13	7.69	0.00	2.00
113	Simmersted	1990	525.00	6128.50	6146.80	0.00	2.00	0.03	32.00	0.00	4.00		
181	Kastvra	2000	521.36	6129.05	6146.90	0.00	3.00	0.00	24.00	0.00	6.00		
182	Kastvra A	2000	522.15	6129.22	6147.17	0.00	16.00	0.01	174.00	0.04	26.00		
187	Sillerup	2000	535.74	6128.38	6147.29	0.00	8.76	0.06	43.00	0.07	9.02		
189	Slusen	2000	538.15	6133.66	6152.34	0.00	1.00	0.00	10.00	0.50	2.00		
92	Oedis	1984	524.40	6140.50	6158.64	0.56	4.84	0.12	28.00	0.64	3.50	0.00	6.00
88	Maltbaek Mark	1992	505.10	6144.75	6160.50	0.00	3.48	0.13	40.00	0.33	2.70		
93	Oedis F	1985	526.30	6142.30	6160.66	0.00	1.50	0.13	18.00	0.50	1.50		
87	Maltbaek	1992	504.10	6145.25	6160.88	0.00	9.01	0.11	107.00	0.00	11.93	0.00	7.00
184	Lauritzminde	2000	541.41	6144.14	6161.99	0.00	26.00	0.00	194.00	0.45	36.47		
175	Binderup	2000	535.09	6143.27	6162.19	0.00	4.00	0.00	34.00	0.00	4.00		
188	Skartved	2000	534.22	6145.88	6164.84	0.00	2.00	0.04	24.00	0.00	2.00		
190	Soender Stenderup	2000	538.65	6146.51	6164.95	0.00	1.00	0.00	12.00	0.00	2.00		
174	Baeklund	2000	516.18	6149.29	6166.36	0.00	12.04	0.05	57.00	0.05	6.76		
179	Gelballe	2000	522.60	6149.29	6167.14	0.20	5.00	0.00	46.00	0.14	7.00		
154	Vranderup	1998	524.80	6149.75	6167.87	0.00	3.09	0.19	20.00	0.33	2.52	0.00	1.00
33	Gesten	1998	511.20	6154.70	6171.12	0.00	1.91	0.28	9.00	0.00	1.17	0.00	1.00
133	Trelde	1998	549.10	6162.30	6172.89	0.00	1.28	0.14	9.00	0.50	1.28		
140	Vejlby	1998	545.40	6162.95	6176.24	0.00	1.97	0.14	25.00	0.00	3.87	0.00	1.00
10	Baekke	1998	509.70	6160.60	6176.80	0.13	5.95	0.21	31.00	0.04	3.95	0.00	8.00
151	Viuf	1998	531.70	6158.85	6177.74	0.00	6.22	0.14	36.00	0.06	4.57	0.00	5.00
153	Vorbasse sk	1992	507.10	6162.20	6178.07	0.00	1.00	0.06	16.00	0.00	2.00	0.00	1.00
101	Rands A	1998	543.65	6164.20	6178.38	0.00	2.00	0.00	6.00	0.00	2.00		
152	Vorbasse	1992	503.50	6163.15	6178.57	0.00	4.88	0.25	22.00	0.16	2.79	0.00	8.00
100	Rands	1998	543.40	6164.75	6178.93	0.00	2.66	0.23	21.00	0.00	2.66		
124	Starup	1998	487.10	6166.80	6180.20	1.00	1.00	0.19	10.00	0.00	1.23	0.00	1.00
24	Egtved 1	1990	519.50	6163.30	6180.67	0.00	1.00	0.19	12.00	0.00	1.45		
	Egtved	1991	522.00	6164.25	6181.92	0.00	1.00			0.00	8.00		

APPENDIX Continued

No.	Locality	Year	Longitude	Latitude	Transect	<i>Btk</i>		Average autosomal locus		<i>Abpa</i>		<i>Zfy</i>	
						fr <i>mus</i>	<i>N_e</i>	fr <i>mus</i>	<i>N_e</i>	fr <i>mus</i>	<i>N_e</i>	fr <i>mus</i>	<i>N</i>
7	Ammistbol	1990	525.60	6165.50	6183.60	0.00	2.55	0.25	16.00			0.00	1.00
128	Toerskind	1984	519.88	6166.90	6184.29	0.00	8.42	0.10	69.00	0.00	7.90	0.00	9.00
129	Toerskind	1985	519.88	6166.90	6184.29	0.50	3.62	0.10	46.00	0.10	5.51	0.00	4.00
130	Toerskind	1991	519.88	6166.90	6184.29	0.00	7.81	0.20	28.00	0.04	5.89	0.00	4.00
8	Ammistbol A	1992	527.30	6166.50	6184.80			0.25	10.00				
137	Tufkaer Mark	1998	531.60	6166.05	6184.87	0.00	1.00	0.21	9.00	0.00	1.28		
95	Oedsted	1990	523.90	6167.25	6185.13			0.44	7.00	0.00	0.84		
94	Oedsted	1991	523.90	6167.25	6185.13	0.00	1.37	0.16	16.00	0.00	2.09	0.00	2.00
96	Oedsted	1992	523.90	6167.25	6185.13	0.00	8.79	0.20	46.00	0.04	6.21	0.00	8.00
14	Bindeballe	1992	517.60	6168.30	6185.40	0.14	4.39	0.23	31.00	0.00	3.94	0.00	2.00
	Stubberup	1990	528.50	6167.05	6185.49	0.00	2.00			0.00	2.00		
	Mejsling	1998	526.90	6168.70	6186.93	0.00	1.00			0.00	2.00		
117	Skoldbjerg	1992	505.85	6171.85	6187.49	0.05	8.97	0.20	51.00	0.12	6.89		
52	Hojen Kirke	1998	531.00	6168.90	6187.63	0.00	6.93	0.18	33.00	0.04	5.30	0.00	3.00
72	Jerlev V	1992	527.45	6170.20	6188.49	0.17	7.64	0.09	49.00	0.00	6.26	0.00	5.00
71	Jerlev A	1998	528.00	6170.25	6188.60	0.00	3.87	0.12	24.00	0.00	3.01		
118	Smakaer	1998	520.40	6171.20	6188.62	0.00	1.88	0.34	27.00	0.25	3.56	0.50	2.00
134	Troellund	1992	502.50	6173.80	6189.02	0.00	6.79	0.26	36.00	0.21	4.41	0.00	7.00
102	Ravning	1992	521.85	6171.55	6189.15	0.00	8.74	0.41	58.00	0.05	7.69	0.00	2.00
125	Store Lihme	1987	519.70	6172.30	6189.63	0.40	3.92	0.38	43.00	0.75	2.80	1.00	3.00
99	Plouglund A	1992	505.90	6174.04	6189.67	0.00	7.03	0.29	39.00	0.23	5.13	0.00	4.00
98	Plouglund	1992	505.95	6174.05	6189.69	0.00	7.47	0.26	38.00	0.21	4.86	0.00	12.00
70	Jerlev	1998	528.00	6171.70	6190.04	0.00	1.00	0.64	13.00	0.00	1.80	0.00	1.00
73	Kaerbolling	1987	525.40	6172.70	6190.72	1.00	0.75	0.57	5.00	0.50	0.75		
145	Vilstrup	1992	529.35	6172.30	6190.80	0.33	3.00	0.27	36.00	0.50	2.00	0.00	1.00
20	Bredsten	1985	523.20	6173.10	6190.85	0.67	3.00	0.52	52.00	0.50	4.00		
21	Bredsten	1987	523.20	6173.10	6190.85	0.67	2.40	0.47	16.00	1.00	2.17	1.00	1.00
	Bredsten	1991	523.20	6173.10	6190.85					1.00	2.00		
26	Englesholm Mark	1998	521.10	6173.55	6191.04	0.00	1.00	0.56	8.00	0.50	1.06	1.00	1.00
13	Balle A	1987	523.70	6174.10	6191.90	1.00	1.00	0.44	16.00	1.00	1.94	0.00	1.00
44	Grene Kirke	1998	504.40	6176.50	6191.93	0.20	4.60	0.33	27.00	0.33	3.38	0.00	1.00
11	Balle	1992	522.80	6174.65	6192.34	0.65	11.85	0.60	110.00	0.43	14.14	1.00	6.00
12	Balle	1998	522.80	6174.65	6192.34	0.00	1.00	0.64	14.00	0.75	2.44	1.00	1.00
120	Soedover	1985	521.55	6174.95	6192.48	1.00	2.80	0.50	64.00	1.00	3.29	1.00	2.00
121	Soedover	1992	521.55	6174.95	6192.48	1.00	1.00	0.50	12.00	1.00	1.46		
80	Lildfrost	1998	525.90	6174.80	6192.87	0.67	2.28	0.56	14.00	0.25	1.88		

15	Braendgaarde	1998	512.20	6176.70	6193.08	0.50	2.24	0.67	15.00	1.00	1.93	1.00	2.00
45	Grinsted	1992	497.85	6178.90	6193.52			0.88	15.00				
142	Vester Hornstrup	1998	526.20	6175.90	6193.99	0.51	8.68	0.47	39.00	0.61	4.68	1.00	17.00
138	Uhre	1998	531.60	6175.60	6194.35	0.75	1.27	0.63	10.00	1.00	1.27		
143	Vester Hornstrup A	1998	526.80	6176.20	6194.36	1.00	1.00	0.50	11.00	1.00	1.77	1.00	1.00
31	Gammelby	1992	520.75	6177.35	6194.77	0.00	1.00	0.58	35.00	1.00	2.00	1.00	2.00
32	Gammelby	1998	520.75	6177.35	6194.77	1.00	1.32	0.69	11.00	1.00	1.32		
25	Elkaer	1998	504.80	6179.50	6194.96	1.00	1.00	0.81	11.00	1.00	1.33	1.00	1.00
106	Rugballe	1992	527.80	6176.80	6195.08	0.18	5.67	0.53	37.00	0.93	4.14	1.00	7.00
107	Rugballe A	1992	527.70	6177.00	6195.27	1.00	1.03	0.69	8.00	1.00	1.39	1.00	1.00
22	Brunbjerg	1998	502.80	6180.10	6195.31	1.00	1.00	0.69	8.00	0.50	0.97	1.00	1.00
108	Rugballe B	1991	528.00	6177.05	6195.35	1.00	4.75	0.71	26.00	1.00	2.86		
109	Rugballe B	1992	528.00	6177.05	6195.35	0.33	2.29	0.60	18.00	0.75	1.90	0.50	2.00
110	Rugballe B	1998	528.00	6177.05	6195.35	0.50	1.11	0.58	14.00	0.50	1.61	1.00	1.00
61	Hovertoft	1998	532.50	6176.50	6195.36	1.00	2.32	0.75	17.00	0.75	1.94		
60	Hover Kirke A	1998	531.00	6176.70	6195.37	1.00	1.00	0.63	8.00	1.00	0.94	1.00	1.00
81	Lille Hoegsholt	1998	531.00	6176.75	6195.42	1.00	1.00	0.88	16.00	1.00	2.00	1.00	1.00
59	Hover Kirke	1998	529.90	6177.10	6195.64	0.67	2.53	0.69	19.00	1.00	2.47		
97	Oelgod	1998	478.85	6184.00	6196.26	0.00	1.19	0.31	10.00	0.00	1.19		
135	Trollerup	1992	522.70	6178.70	6196.35	1.00	1.58	0.46	23.00	0.75	2.60	1.00	2.00
136	Trollerup	1998	522.70	6178.70	6196.35	1.00	2.69	0.72	22.00	1.00	2.92	1.00	1.00
82	Lindeballe	1992	512.25	6180.30	6196.66	0.55	5.05	0.61	23.00	0.41	3.03	1.00	5.00
67	Jelling bis	1987	526.10	6178.80	6196.86	1.00	1.00	0.88	21.00	1.00	2.14		
83	Lindeballe A	1992	513.00	6180.65	6197.10	1.00	1.00	0.44	9.00				
74	Kiddelund	1998	522.90	6179.50	6197.16	0.75	2.66	0.80	18.00	0.83	2.39	1.00	2.00
89	Moelvang	1990	522.85	6179.60	6197.26	0.67	2.72	0.50	22.00	1.00	1.72		
90	Moelvang	1992	522.85	6179.60	6197.26	0.50	1.10	0.75	9.00	1.00	1.10		
	Moelvang	1991	522.85	6179.60	6197.26	1.00	1.00			1.00	2.00		
66	Jelling	1990	526.45	6179.25	6197.35	0.75	3.04	0.82	20.00	0.67	3.09		
50	Hoerup	1990	530.15	6179.50	6198.05	0.64	2.64	0.67	25.00	0.70	2.79		
51	Hoerup	1991	530.15	6179.50	6198.05	0.69	4.52	0.71	20.00	0.92	2.49	1.00	14.00
48	Hoegelund	1998	509.80	6182.10	6198.15	0.92	12.79	0.67	95.00	0.95	10.96	0.83	6.00
47	Hjortlund	1998	504.20	6182.80	6198.16	0.25	10.11	0.64	64.00	0.91	9.34	1.00	3.00
30	Gadbjerg	1987	520.50	6181.20	6198.56	0.50	0.87	0.67	5.00	1.00	0.87		
91	Norskov	1992	515.30	6182.05	6198.77	1.00	1.20	0.66	13.00	0.50	1.20	1.00	1.00
78	Langelund S	1998	508.25	6183.15	6199.00	1.00	4.68	0.56	24.00	0.79	2.92	1.00	9.00
131	Toftthoj	1987	521.20	6181.70	6199.14	1.00	1.14	0.50	8.00	0.50	1.14		
119	Smidstrup	1992	516.85	6182.35	6199.26	0.50	5.20	0.69	42.00	0.50	5.18	1.00	2.00
29	Filskov A	1992	500.10	6184.50	6199.35	1.00	1.62	0.81	13.00	0.50	1.62		
16	Brandbjerg	1985	529.30	6181.15	6199.58	1.00	3.88	0.82	32.00	0.67	2.32		
17	Brandbjerg	1991	529.30	6181.15	6199.58	0.74	16.10	0.85	85.00	0.94	10.95	1.00	19.00
18	Brandbjerg	1992	529.30	6181.15	6199.58	1.00	1.00	0.69	7.00	1.00	0.88		
63	Hygum	1990	527.40	6181.40	6199.60	1.00	1.00	0.81	10.00	1.00	1.27		
64	Hygum A	1998	526.00	6181.60	6199.63	1.00	2.91	0.91	29.00	1.00	3.67	1.00	1.00

APPENDIX Continued

No.	Locality	Year	Longitude	Latitude	Transect	Btk		Average autosomal locus				Abpa		Zfy			
						fr	mus	fr	mus	N _e	N _e	fr	mus	N _e	fr	mus	N
28	Filskov	1992	499.20	6185.05	6199.78	1.00	2.35	0.78	15.00	0.50	2.04						
19	Bredal	1998	538.00	6179.30	6199.86	0.40	3.70	0.69	31.00	0.88	3.82	1.00		2.00			
77	Langelund F	1998	504.25	6184.80	6200.15	1.00	4.47	0.74	27.00	1.00	3.61						
65	Hygum B	1998	527.70	6182.60	6200.83	0.92	7.71	0.86	40.00	1.00	4.98	1.00		5.00			
146	Vindelev M	1985	530.30	6182.60	6201.14	1.00	1.00	0.73	41.00	1.00	1.64	1.00		3.00			
147	Vindelev M	1987	530.30	6182.60	6201.14	1.00	1.00	0.86	11.00	1.00	1.60	1.00		1.00			
148	Vindelev M	1991	530.30	6182.60	6201.14	1.00	4.14	0.69	23.00	0.64	4.99	1.00		2.00			
149	Vindelev S	1991	530.05	6182.70	6201.21	0.96	4.49	0.74	21.00	0.80	2.82	1.00		6.00			
150	Vindelev S	1992	530.05	6182.70	6201.21	1.00	7.48	0.66	43.00	0.71	5.26	1.00		13.00			
111	Sandvad	1990	528.00	6183.45	6201.71	0.92	5.05	0.79	27.00	0.75	3.32	1.00		4.00			
112	Sandvad	1991	528.00	6183.45	6201.71	1.00	1.98	0.63	8.00	1.00	0.98						
84	Lindved	1990	536.10	6183.00	6202.37	1.00	2.91	0.83	25.00	1.00	2.29	1.00		2.00			
103	Riis A	1998	520.10	6185.50	6202.78	1.00	2.75	0.69	14.00	1.00	1.89	1.00		1.00			
36	Givskud Z	1987	522.00	6185.40	6202.91	1.00	3.53	0.86	32.00	1.00	3.53	1.00		1.00			
37	Givskud Z	1991	522.00	6185.40	6202.91	0.68	15.34	0.75	93.00	0.70	11.93	0.17		6.00			
38	Givskud Z	1998	522.00	6185.40	6202.91	1.00	9.82	0.80	45.00	0.87	5.86	1.00		12.00			
35	Givskud E	1991	521.95	6185.95	6203.45	1.00	3.29	0.84	19.00	0.75	1.77	1.00		2.00			
2	Aadel A	1991	528.70	6185.40	6203.73	1.00	1.03	0.44	8.00	1.00	1.03						
3	Aadel A	1998	528.70	6185.40	6203.73	1.00	3.60	0.71	34.00	1.00	5.53	1.00		1.00			
62	Hvejsel	1998	526.45	6185.70	6203.75	0.33	3.68	0.81	24.00	0.88	3.12	1.00		1.00			
9	Baastруп	1987	540.50	6182.55	6203.96	0.86	6.43	0.77	39.00	0.65	4.86	1.00		7.00			
34	Give Mark	1992	514.40	6187.45	6204.02	1.00	4.74	0.88	33.00	1.00	3.74	1.00		2.00			
116	Skaerhoved	1998	517.20	6187.40	6204.31			0.75	14.00	1.00	1.69						
1	Aadel	1990	529.00	6186.00	6204.36	1.00	2.41	0.81	21.00								
122	Soendersthoved	1998	510.50	6188.30	6204.39	1.00	3.20	0.81	22.00	1.00	2.91	1.00		3.00			
104	Riis	1998	519.90	6187.40	6204.64	1.00	4.92	0.81	41.00	0.75	5.22	1.00		2.00			
144	Vibjerg	1998	517.70	6188.10	6205.07	1.00	1.00	0.88	16.00	0.50	1.99	1.00		1.00			
	Fousing	1990	528.65	6186.97	6205.28	1.00				1.00	2.00						
46	Hedegaard	1998	510.80	6189.20	6205.32	1.00	1.00	0.88	16.00	1.00	2.00	1.00		1.00			
4	Aalsted Moelle	1991	529.20	6187.20	6205.57	1.00	3.02	0.80	14.00	0.92	1.98	1.00		1.00			

6	Aalsted Moelle	1992	529.20	6187.20	6205.57	0.92	7.58	0.76	45.00	0.94	6.17	1.00	3.00
105	Riis Mark	1998	521.30	6188.40	6205.80	1.00	14.18	0.78	103.00	0.92	13.90	1.00	4.00
85	Loesning	1984	544.50	6184.50	6208.05	0.86	5.33	0.78	54.00	0.79	7.72	1.00	6.00
86	Loesning	1985	544.50	6184.50	6208.05	0.80	3.83	0.85	48.00	1.00	2.71	1.00	2.00
127	Toerring Mark	1998	532.80	6192.20	6210.98	0.89	9.00	0.81	78.00	0.75	12.00	1.00	4.00
126	Thyregod	1998	520.40	6195.00	6212.24	1.00	1.81	0.63	26.00	1.00	3.30	1.00	2.00
53	Honum	1998	539.65	6193.50	6213.45	1.00	5.79	0.81	24.00	0.78	3.09	1.00	12.00
	Hov	1984	576.60	6197.85	6241.93							1.00	12.00
76	Klovborg	1998	532.55	6198.15	6216.85	1.00	1.36	0.86	9.00	1.00	1.36		
27	Estrupholm	1998	518.43	6203.85	6220.79	1.00	2.83	0.94	26.00	1.00	3.39	1.00	1.00
139	Underup	1998	542.95	6200.57	6221.20	1.00	14.01	0.87	69.00	1.00	9.00	1.00	10.00
132	Traeden	1998	542.02	6202.65	6222.89	1.00	2.66	0.80	31.00	1.00	4.79	1.00	1.00
141	Vestbirk	1998	545.50	6202.45	6223.86	1.00	5.18	0.80	23.00	0.79	2.82	1.00	10.00
	Oestbirk	1991	547.50	6202.50	6224.73	1.00	7.00						
178	Gammel Hampen	2000	523.26	6207.23	6224.73	1.00	10.04	0.92	50.00	1.00	9.54		
196	Vester Gludsted	2000	518.02	6209.32	6226.17	1.00	0.83	0.75	5.00				
192	Store Noerlund	2000	514.92	6210.51	6226.97	1.00	4.42	0.96	22.00	1.00	3.65		
	Langbanke	2000	518.56	6211.84	6228.73	1.00	1.00			1.00	2.00		
49	Hoelby	1992	575.40	6197.40	6240.67	1.00	12.81	0.95	107.00	1.00	13.22	1.00	5.00
39	Gosmer F	1991	574.50	6199.10	6240.78	1.00	11.00	0.97	112.00	1.00	10.00	1.00	3.00
	Gosmer F	1992	574.50	6199.10	6240.78	1.00	13.00			1.00	4.00		
41	Gosmer N	1991	574.65	6199.75	6241.26	1.00	9.36	0.94	45.00	1.00	5.81	1.00	8.00
42	Gosmer N	1992	574.65	6199.75	6241.26	1.00	6.88	0.90	34.00	0.73	4.38	1.00	8.00
43	Gosmer N	1998	574.65	6199.75	6241.26	1.00	13.03	0.87	57.00	1.00	10.09	1.00	7.00
180	Gosmer N	2000	574.65	6199.75	6241.26	1.00	6.20	0.96	26.00	1.00	5.69		
40	Gosmer J	1991	575.70	6198.75	6241.61	1.00	7.00	0.96	80.00	1.00	10.00	1.00	3.00
55	Hov	1985	576.60	6197.85	6241.93	1.00	2.43	0.99	50.00	1.00	4.08	1.00	2.00
56	Hov	1987	576.60	6197.85	6241.93	1.00	8.98	0.96	81.00	1.00	8.77	1.00	7.00
57	Hov	1991	576.60	6197.85	6241.93	1.00	4.00	1.00	32.00	1.00	8.00	1.00	1.00
58	Hov	1992	576.60	6197.85	6241.93	1.00	2.93	0.83	19.00	0.75	2.49		
	Gosmer VN	1991	576.00	6199.00	6242.00	1.00	9.00			1.00	18.00		
123	Spoettrup	1998	577.50	6199.20	6243.39	1.00	15.90	0.93	175.00	1.00	27.51	1.00	10.00
191	Spoettrup	2000	577.50	6199.20	6243.39	1.00	7.79	0.96	100.00	0.94	14.71		
177	Elleskovhuse	2000	552.29	6223.20	6245.66	1.00	9.83	0.95	45.00	1.00	6.93		
75	Klank	1987	556.80	6223.20	6247.42	1.00	5.25	0.93	35.00	1.00	3.83	1.00	4.00
194	Tovstrup	2000	550.54	6226.03	6247.75	1.00	1.00	1.00	2.00	1.00	2.00		
197	Voel Oestermark	2000	545.54	6228.33	6248.65	1.00	3.00	1.00	14.00	1.00	4.00		
173	Abo	2000	564.51	6220.48	6248.81	1.00	8.74	0.96	61.00	1.00	9.77		
185	Ormslev	2000	566.71	6220.50	6250.07	1.00	19.00	0.97	38.00	1.00	24.00		
176	Borum Oestergaard	2000	563.90	6227.26	6254.29	1.00	5.87	0.96	32.00	1.00	6.50		