nature genetics

Genetic similarity and quality interact in mate choice decisions by female mice

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Females express mate preferences for genetically dissimilar males[1], especially with respect to the major histocompatibility complex, MHC[2,3], and for males whose sexually selected signals indicate high genetic quality[4,5]. The balance of selection pressure on each trait will depend on how females weight these desirable qualities under different conditions[6], but this has not been tested empirically. Here we show in mice that although MHC dissimilarity and a 'good genes' indicator (investment in scent-marking) both have a role in determining female preference, their relative influence can vary depending on the degree of variability in each trait among available males. Such interactions between condition-dependent and disassortative mate choice criteria suggest a mechanism by which female choice can contribute to maintenance of additive genetic variance in both the MHC and condition-dependent traits, even under consistent directional selection.

Mice are known to discriminate between individuals with similar or dissimilar genotype at the MHC (H-2 in mice; refs. 7,8). Females prefer MHC-dissimilar over MHC-similar males[2,3], resulting in greater offspring heterozygosity and viability[3,9]. Cues regarding MHC similarity are mediated by urinary odor[10]. Simultaneously, androgen-dependent urinary odor cues advertise male status and are thought to be 'good genes' indicators of quality[11]. Urinary scent marks differ qualitatively between dominant and subordinate males[12] and are produced at higher frequencies in dominants[13,14]. Urinary scent-marking is costly in terms of reduced growth and smaller asymptotic size[13], and marking at high frequency by dominant males may thus be seen as 'honest' signaling of quality[15]. Females prefer the odor of dominant over subordinate males[16,17] and prefer males whose marking patterns suggest they are of high quality[18]. Furthermore, rodent urinary scent-marking rates and associated gland sizes are heritable[19] and sons of high-quality males are preferred by females[20], indicating indirect fitness benefits of preferences based on these qualities. Although the evidence for each of these two effects is thus extensive, the interrelationship between them is unknown, even though they stem from the same urinary signal.

To investigate the interaction between these cues, we used five strains of MHC-congenic laboratory mice (B10.A and B10.A(2R), (3R), (4R) and (5R)). Different pairs of strains shared 1–11 alleles at 12 H-2 loci (**Table 1**). We measured the scent-marking rates of males from each strain, and these varied significantly between strains (ANOVA, $F_{4,67} = 2.54$, $P < 0.05$; **Fig. 1**).

We assessed the preferences of females from each strain for males from each of the four other strains in a four-way choice test. For each female, we ranked the males in order of preference and, using the most- and least-preferred males, compared this ranking with each strain's marking rate and genetic distance. Using ranked variables in each case, female preference was correlated positively with scent-marking rate (Kendall's tau-b = 0.338, $n = 79$, $P < 0.001$) and negatively with the number of shared alleles (tau-b = –0.198, $n = 79$, $P = 0.048$). These results are consistent with previous studies that show that each trait predicts preference when considered in isolation. To test the relative importance of these effects on female preferences, we carried out a logistic regression analysis, with preference rank as the dependent variable and with ranked genetic distance and ranked marking rate as factors. Female preference was predicted independently by scent-marking rate ($\chi^2 = 9.73$, d.f. = 1, $P = 0.002$) but not by genetic distance ($\chi^2 = 1.81$, d.f. = 1, $P = 0.179$).

As mice can nonetheless discriminate between potential mates of variable dissimilarity at the MHC, there are probably appropriate circumstances in which this ability is adaptive. We hypothesized that

Table 1 Haplotypes of the five H-2 congenic strains

Strain	Loci											
	K	Aβ	Aa	Eβ	Ea	C4	C4S	D	Qa-2	Tla	Qa-1	J
B10.A	k	k	k	k	k	d	d	D	a	a	a	k
B10.A(2R)	k	k	k	k	k	d	d	B	a	b	b	k
B10.A(3R)	b	b	b	b/k	k	d	d	D	a	a	a	b
B10.A(4R)	k	k	k	k/b	b	b	b	B	a	b	b	b
B10.A(5R)	b	b	b	b/k	k	d	d	D	a	a	a	k

Haplotype data were obtained from the supplier, Harlan UK.

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Published online 24 August 2003; doi:10.1038/ng1231

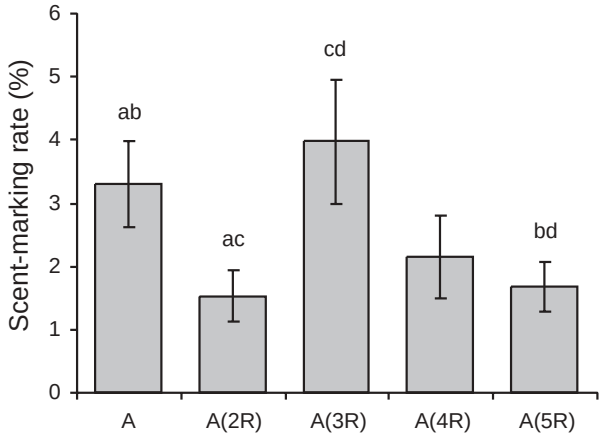

Figure 1 Scent-marking rates (mean ± s.e.) of males from five MHC-congenic B10 mouse strains. Variation between strains is significant ($P < 0.05$); common letters indicate post-hoc significant differences.

it might be most useful when there was little variation in marking rates to choose from or, alternatively, when there was a great deal of variation in genetic distance. Similarly, marking rate might be more important as a predictor of mate preferences when variation in marking rate was very high or when variation in genetic distance was limited. To test this, we repeated the analyses after splitting the strains into those in which females could choose between a relatively small or large range of genetic distances and scent-marking rates (**Table 2**). When considering bivariate relationships, we found that marking rate was consistently correlated with preference but that genetic distance was important only when the range of choices for male marking rates was limited (**Table 3a**). Further, binomial logistic regressions showed a marked pattern of predictive value for female preferences. Marking rate consistently predicted preference independently of genetic dissimilarity, whereas dissimilarity predicted preference only when variability in dissimilarity among the males was relatively large, or conversely, when the variability in marking rates was small (**Table 3b**).

Our results draw together two extensive, but so far separate, bodies of literature on the influence of male genetic quality and MHC dissimilarity on female preferences among potential mates and begin to address the question of how females weight these cues when making a choice. In these MHC-congenic strains, the significant association between MHC type and scent-marking rate suggests that loci within the MHC may encode, or have pleiotropic effects on, this phenotypic trait. Although a similar association between MHC genotype and spur length (a condition-dependent trait used in female choice) in pheasants, *Phasianus colchicus*, has been previously reported[21], our use of congenic strains enables us to control for differences in genetic background[22].

At least in the strains we used, marking rate predicts female preference more reliably than MHC dissimilarity. This preference persisted, despite the fact that the strains used were highly inbred, a circumstance in which one might expect inbreeding avoidance to be of paramount importance. It is possible that scent-marking is a more reliable cue of male genetic quality than MHC similarity is of genetic relatedness (assuming some degree of MHC similarity exists by chance between non-related individuals) and, thus, that choice is biased towards the more reliable cue. Another possibility is that the outcome of this interaction might be determined not directly by trait characteristics themselves but by better resolution, and thus more effective discrimination, in one trait than the other. Evidence against the latter suggestion includes the findings that females were able to discriminate on the basis of both cues under defined circumstances, that the coefficient of variation was much higher for similarity (34%) than marking rate (13%) and that MHC-disassortative preferences have previously been reported in B10.A mice[23]. It seems, therefore, that both components of urinary signals have important, though unequal, roles in mate choice. Each component signals separate, sometimes conflicting, adaptive value, raising the possibility of trade-offs between them within the decision-making process. Evidence for this comes from the multivariate analyses when the sample is split according to variability in marking rate and genetic similarity among the males.

Similar interactions between condition-dependent and disassortative mate choice criteria probably exist in other mouse strains and in other species. If so, this carries a number of implications for interpretation of

Table 2 Variation in genetic similarity and marking rate among males assessed by females

Female strain	Male strain					Range	Category
	A	2R	3R	4R	5R		
(**a**) Number of shared alleles							
A	–	9	7	4	8	4–9	Small
2R	9	–	4	7	5	4–9	Small
3R	7	4	–	2	11	2–11	Large
4R	4	7	2	–	1	1–7	Small
5R	8	5	11	1	–	1–11	Large
(**b**) Scent-marking rate (% cover)							
A	–	1.53	3.97	2.22	1.68	1.53–3.97	Large
2R	3.3	–	3.97	2.22	1.68	1.68–3.97	Small
3R	3.3	1.53	–	2.22	1.68	1.53–3.3	Small
4R	3.3	1.53	3.97	–	1.68	1.53–3.97	Large
5R	3.3	1.53	3.97	2.22	–	1.53–3.97	Large

Amount of variation in genetic similarity and marking rate among males assessed by females from each strain. On the basis of this variation, strains were categorized as those in which females had a relatively small or large available range from which to choose.

Table 3 Effects of male quality and dissimilarity on female preference

		Variation in scent-marking rate		Variation in MHC dissimilarity	
		Small	Large	Small	Large
(**a**) Kendall's tau-b					
Scent-marking rate (% cover)	tau-b	0.422	0.297	0.338	0.366
	n	31	48	48	31
	P	0.003	0.014	0.005	0.013
Number of shared alleles	tau-b	–0.347	–0.091	–0.162	–0.252
	n	31	48	48	31
	P	0.020	0.492	0.212	0.115
(**b**) Logistic regression					
Scent-marking rate (% cover)	χ^2_1	5.55	5.29	6.90	8.40
	P	0.018	0.021	0.009	0.004
Number of shared alleles	χ^2_1	4.30	0.08	0.0	5.70
	P	0.038	0.778	0.996	0.017

Effects of male quality (scent-marking rate) and dissimilarity on female preference when there is large or small variability in each cue for females to choose between. (**a**) Kendall's tau-b correlations. (**b**) Binomial logistic regressions.

mate-choice studies. First, it shows the complexity of decision-making, with individuals simultaneously perceiving both kinds of cue and processing trade-offs between them. A female's final choice will be determined by the relative fitness benefits resulting from choosing the highest quality male, as indicated by condition-dependent signaling, or those resulting from choosing the most MHC-dissimilar male. This trade-off has recently been modeled[6], and we show here that it is influenced by the expressed variability in these traits between available males. Second, the complex nature of such interactions offers a plausible explanation for discrepancies between studies in the importance of MHC-mediated mating preferences. Although a growing number of studies in mice and humans show disassortative preferences[3,24,25], others detect no effect[24,26]. Our results suggest the possibility that, in such cases, this is due to greater influence of condition-dependent cues or limited MHC variation between males.

Finally, our results contribute to the debate concerning the role of mating preferences in the evolution and maintenance of diversity both in the MHC (the 'holy grail of MHC biology'; refs. 27,28) and in expression of condition-dependent traits (the lek paradox[29,30]). Complex patterns of mate preference, involving context-dependent decision-making and possible trade-offs between genetic quality and dissimilarity, may result in discrepancies between females in terms of ideal mate choice[6]. These discrepancies, coupled with our finding in these congenic strains that MHC genotype is correlated with scent-marking investment, suggest a mechanism by which female choice could contribute to maintenance of variability in both the MHC and condition-dependent sexually selected traits, even under consistent directional selection.

METHODS

Mouse husbandry. We bred ten male-female pairs from each of the five congenic strains (obtained from Harlan UK) in MB1 (45 cm × 28 cm × 13 cm) cages, fed them with *ad libitum* food and water under a reversed 12 h:12 h light:dark cycle at constant temperature (21 ± 2 °C). Litters were weaned at 3 weeks of age and segregated by sex. At 5 weeks of age, we randomly selected 15 males from each strain for this experiment. They were isolated in M2 (20 cm × 10 cm × 13 cm) cages and remained isolated until the preference tests in weeks 15 and 16. Husbandry and all research procedures were carried out under license from the United Kingdom Home Office, in compliance with the Animals (Scientific Procedures) Act 1986.

Measuring marking rates. We measured scent-marking rates of each male weekly between 6 and 15 weeks of age. We placed a plastic block (5 cm × 5 cm × 4 cm) covered by clean Benchkote absorbent paper in the cage for 1 h at the beginning of the dark cycle. Mice regularly scent-mark novel objects in their environment, enabling assessment of individual variation in the intensity of scent-marking[13]. We used ninhydrin to stain the scent marks deposited by males on the paper and quantified the percentage of the paper covered by the marks (% cover) using an overlaid transparent grid. We calculated mean marking rates and discarded one outlier (>3 s.d. from the mean) from each of three strains. We arcsine-transformed data before testing with univariate analysis of variance and used least-significant difference post-hoc tests to determine which strains marked at different rates from each other.

Preference tests and analyses. We measured female preference for males from each of the four other strains in a choice test. We placed estrus females ($n = 8$ per strain) into a central chamber connected by 15-cm tubes to four end-chambers. Males were placed behind a perforated screen within the end-chambers 10 min before the females were introduced. Females could not see the males from the central cage, nor could males see each other. Mesh caps at the distal ends of the tubes prevented females from entering the end-chambers. We recorded the tests on video and recorded the time females spent in each tube over a 10-min period. Two females did not leave the central chamber and were excluded from the analyses. All remaining 38 females visited at least one male; 35 visited every male at least once, one visited three males, one visited two males and one visited only one male (because of tied ranks among males not visited by females, $n = 79$, not 76, in our analyses). We ranked female preference for each male based on time spent in each tube.

In addition, we used ranked marking rate and genetic distance in all analyses. We used strain marking rate, rather than individual marking rate, because there was greater variation between strains than between individuals and to equalize statistical power in comparisons of the effects of marking rate and genetic distance (which, by definition, did not vary across individuals from the same strain). We checked for effects of ranked individual marking rates on female preference but did not find any (Kendall's tau-b = 0.007, $n = 79$, not significant), probably owing to greater measurement error when measuring individual rates than when calculating mean marking rates of strains.

We also checked that females of different strains did not behave differently in terms of spending time in tubes (one-way ANOVA, $F_{4,33} = 0.254$, $P = 0.91$). In all analyses presented, we used data from the most- and least-preferred males, excluding the intermediate two males. We did this primarily because it avoided a statistical violation (low singularities in the Hessian matrix, indicating some categories should be merged) encountered if data from all males were analyzed by multinomial logistic regression.

In the last analysis, we tested whether effects were influenced by the degree of variation that was available for females to choose from in either marking rate or genetic distance. We first subdivided the data (**Table 2**) according to whether females were from strains of intermediate scent-marking rate (or had the largest possible variation from which to choose) or from strains with the lowest or highest marking rates (or had a narrower range from which to choose). We used a similar approach to compare the effect of degree of variation in genetic distance on female preferences. The number of shared alleles between females and males varied between 2 and 11 and between 1 and 11 for females of strain B10.A(3R) and B10.A(5R), respectively (10–11 alleles shared between females and males), whereas females of strains B10.A, B10.A(2R) and B10.A(4R) shared only 6, 6 and 7 alleles, respectively, with males (see **Table 2**). We checked the robustness of this subdivision by repeating analyses shown in **Table 3** after recategorizing strain B10.A(4R) as a strain having relatively large variation to choose from (including it with B10.A(3R) and B10.A(5R)). Although the exact probability values differ slightly (as expected), the pattern of statistical significance was identical to that presented.

ACKNOWLEDGMENTS

We thank M. Bateson, F. Gilbert, M. Hale, W. Jordan, J. Kotiaho, M. Petrie and C. Rowe for their comments. The work was funded by the Leverhulme Trust.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 2 April; accepted 31 July 2003
Published online at http://www.nature.com/naturegenetics/

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