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SHORT COMMUNICATION

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Agonistic onset during development differentiates wild house mouse males (*Mus domesticus*)

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Abstract Wild house mouse populations have been suggested to locally adapt to varying dispersal regimes by expressing divergent aggressivity phenotypes. This conjecture implies, first, genetic polymorphism for dispersive strategies which is supported by the finding of heritable variation for male dispersal tendency in feral house mice. Secondly, aggressivity is assumed to translate into dispersal rates. This speculation is reinforced by experimental evidence showing that non-agonistic males display lower dispersal propensity than same-aged males that have established agonistic dominance. However, the actual ontogenetic behavioural pattern and its variability among populations remain unknown. Hence, in this study the timing of agonistic onset is quantified within laboratory-reared fraternal pairs, and compared between descendants from two different feral populations. Males from the two populations (G and Z) differed strongly in agonistic development, as Z fraternal pairs had a 50% risk of agonistic onset before 23.5±2.7 days of age, while this took 57.3±5.4 days in males from population G. This difference coincided with significant genetic differentiation between the males of the two populations as determined by 11 polymorphic microsatellite markers. Furthermore, in population G, males from agonistic and amicable fraternal pairs exhibited significant genetic differentiation. These results corroborate the supposition of genetic variability for dispersive strategies in house mice, and identify the ontogenetic timing of agonistic phenotype development as the potential basis for genetic differentiation. This opens a unique opportunity to study the genetic determination of a complex mammalian behavioural syndrome in a life history context, using a simple laboratory paradigm.

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Introduction

Natal dispersal represents one of the most important life history decisions in mammals (Gaines and McClenaghan 1980) and is frequently pre-reproductive and male-biased in rodents (Stenseth and Lidicker 1992), including the house mouse. Most male house mice leave the natal deme during their third month of life, well after reaching sexual maturity (Gerlach 1996). Young males are always subordinate to the territorial male and thought to achieve little reproductive success. However, they will take over the territory if possible (Gerlach 1996). Mature males appear to initiate agonistic interactions with the territory holder before dispersing, thereby probing the potential for becoming territorial and hence reproductive in their own deme; however, if defeated, males are invariably forced to leave the deme due to overwhelming aggression (Gerlach 1996). Hence, it appears that by initiating agonistic interactions a subordinate male determines dispersal from its natal deme, unless it can defeat the territorial male.

Clearly, any evolved decision rule causing initiation of dispersal at a particular age must incorporate estimates of profitability of dispersal as opposed to philopatry (Ims and Hjermann 2001). Since dispersal costs and benefits might strongly vary over time and environments (Clobert et al. 2001), one might suspect genotypes to track these divergencies (Corti and Rohlf 2001). Species might therefore be anticipated to carry polymorphisms regarding the genetically determined components of the behavioural syndrome that is connected to dispersal propensity (Olivieri et al. 1995), though evolutionary theory predicts single global optima under many plausible assumptions (Kisdi 2002).

Empirical findings indicate that genetic polymorphism of dispersal propensity might occur in species like the house mouse. First, data on dispersal of young males from residential populations show that dispersal propensity exhibits heritable variation in wild house mice (*Mus musculus*, Krackow 2003). Furthermore, Corti and Rohlf (2001) argue that dispersal regime variation between alpine house mouse habitats has selected for divergent

dispersal phenotypes. The authors found that mice from populations that are supposed to differ in costs/benefits of dispersal, exhibit significant differences in bite-related morphological characteristics. Concomitantly, mice of different morphological phenotypes differed in standardised aggressivity scores (Corti and Rohlf 2001). The authors, therefore, speculate that these differences in agonistic phenotype might ultimately relate to differences in locally adapted dispersal tendency. In other words, the authors envision a whole suite of morphological, agonistic, and other behavioural characters to represent a syndrome that can be selected for, and locally adapts to variation in dispersal risks and prospects (Corti and Rohlf 2001).

The presumption that agonistic phenotype actually relates to dispersal phenotype has recently been confirmed in our wild house mice (Rusu and Krackow, in press): the onset of agonistic interactions in laboratory-kept fraternal pairs of wild house mice affected dispersal propensity in semi-natural enclosure experiments. When not acquiring territorial status, males that showed agonistic dominance by 2 months of age had a stronger tendency to disperse from a residential group than non-agonistic males. Hence, the onset of agonistic relationships between males of fraternal pairs appears to give a standardised measure of dispersal propensity in male wild house mice (Rusu and Krackow, in press). Consequently, screening for agonistic onset should allow for the investigation of population differentiation of dispersal phenotypes.

In order to get further insights into potential genetic determination of the behavioural syndrome we analysed population differentiation by microsatellite length polymorphisms. We investigated population divergence of the agonistic onset threshold, i.e. the age at which agonistic interactions are initiated, and compared microsatellite length polymorphism patterns between populations as well as between agonistic phenotypes, to differentiate potentially locally adapted dispersal genotypes.

Materials and methods

Experimental animals were offspring from randomly bred descendants of wild-caught house mice (*Mus domesticus*, $2n=24$ chromosomes) from two lowland Swiss locations separated by approximately 40 km (populations G and Z). Parental pairs were randomly assigned and bred monogamously under standard laboratory conditions (perspex Macrolon cages of 26.5 cm×42 cm×15 cm; 12:12 h light/dark cycle with lights on at 0600 hours; 22±1°C; 50–60% relative humidity). Pups were weaned at 21 days of age and placed into similar cages with same-sex littermates, except for the experimental males. All mice of the parental generation were themselves raised under identical conditions.

For the experiment, pairs of littermate males were transferred into Macrolon-cages (22 cm×36 cm×15 cm) at weaning, and then time-lapse video-taped for the dark period every other day. The age at first occurrence of agonistic interactions (cf. Mackintosh 1981, for agonistic behavioural repertoire) was considered to mark the onset of dominance relationships between brothers. Males were separated the day after agonistic onset, i.e. after the first night when agonistic interactions had been observed. Agonistic onset was never doubtful, as initial interactions invariably included conspicuous fleeing and chasing behaviour. Several fraternal pairs remained non-agonistic during the whole study.

Nine breeding pairs of population G contributed 19 fraternal pairs (one to four per breeding pair), and six breeding pairs contributed seven fraternal pairs for population Z. Post-mortem tissue samples (ear lobe cuts) were used to extract DNA by standard alcoholic extraction and to amplify well-known laboratory mouse microsatellite markers (Table 1).

Table 1 Mouse microsatellite markers used, numbers of fragment lengths encountered in populations G, Z, and the total sample. All fragment length distributions differentiated populations G and Z ($P<0.0001$ in each case). *P*-values for genetic differentiation of agonistic and amicable males within population G (cf. text) are also given

Marker	No. of fragments			Agonistic differentiation in G (*P*-level)
	G	Z	Total	
D3Mit12	4	3	4	0.501
D5Mit13	1	1	1	Monomorphic
D5Mit24	2	3	4	0.018*
D5Mit122	3	3	3	0.151
D7Mit266	3	2	4	0.491
D8Mit15	5	3	7	0.020*
D11Mit90	4	3	4	0.374
D13Mit17	4	2	4	0.044*
D13Mit231	3	3	5	0.038*
D17Mit28	5	3	5	0.030*
D17Mit87	3	2	3	0.191
D19Mit25	3	3	5	0.924

* $P<0.05$

Statistical analysis

The age at first occurrence of agonistic interactions or, in the case of non-agonistic fraternal pairs, the age at end of study was entered as dependent variable in a failure-time model (proc Lifereg; SAS Institute 1989). This survival analysis model tested the difference of age at agonistic onset between populations, taking into account the data censoring and assuming Weibull error distribution (i.e. age was entered as dependent variable, population identity as independent class variable, and a dichotomous variable was added that indicated censoring). To avoid spurious results due to pseudoreplication, an additional model was run with a data-set restricted to fraternal pairs that first entered the study per breeding pair (hence, containing nine G fraternal pairs and six Z pairs).

Genetic population differentiation was evaluated by comparison of microsatellite length polymorphisms with GENEPOP (http://www.biomed.curtin.edu.au/genepop). Here, an unbiased estimate of the exact probability for the population×allele contingency is given, i.e. for the probability of identical allelic distribution between populations (Raymond and Rousset 1995). Distributions were compared between populations G and Z, and between mice from population G that did and did not show agonistic onset before 50 days of age. The latter dichotomising criterion was chosen so as to divide the sample into roughly equal portions, and this analysis excluded pairs that could not be classified, i.e. when censoring occurred before 50 days of age.

Results

Fraternal pairs from population G differed significantly in timing of agonistic onset from those of population Z

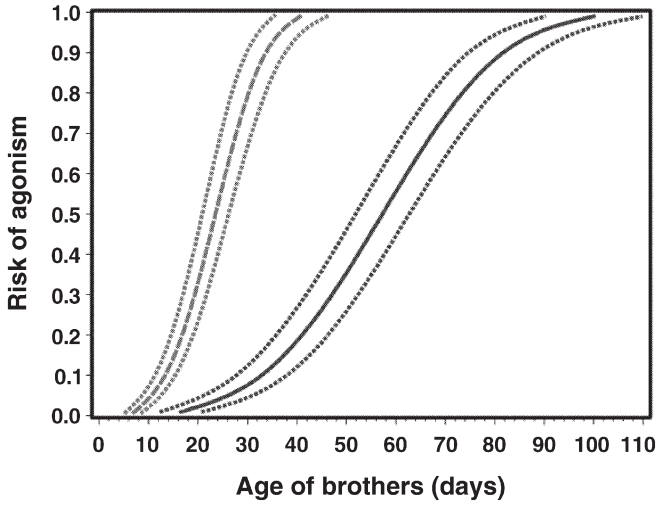

Fig. 1 Risk of agonism to have been initiated as a function of age of fraternal pairs (±SE) for mice from populations G (*solid line*) and Z (*broken line*)

($\chi^2_1 = 39.4$, $P<0.0001$, Fig. 1), a result holding for the restricted data set ($\chi^2_1 = 50.5$; cf. Materials and methods). All seven pairs of brothers from population Z developed agonistic dominance relationships between 23 and 29 days of age, while agonism in the 12 pairs from population G started between 31 and 105 days of age. Seven G-pairs did not exhibit agonism until the end of the experiment. The survival analysis model predicted 50% of Z pairs to engage in agonism by 23.5±2.7 days of age, while agonistic onset half-time was estimated at 57.3±5.4 days of age in G males (cf. Fig. 1).

The experimental males of populations G (n=38 from 19 fraternal pairs) and Z (n=14) exhibited highly significant differentiation at all loci ($P<0.0001$ in all cases). Within population G, males from pairs remaining non-agonistic until at least 50 days of age (n=14) differed significantly from those exhibiting agonism before that age (n=12; overall differentiation: $\chi^2_{22} = 47.8$, $P<0.002$). Five of 11 polymorphic markers differentiated significantly between these two groups of males in population G (Table 1).

Discussion

Timing of agonistic onset differed starkly between maturing house mouse males descending from two feral populations: age at 50% risk of prior agonistic onset within fraternal pairs from population G was roughly double that in population Z. As mice from both populations, including the breeding pairs used in the current study, had been reared under identical laboratory conditions, genetic causes of this divergence seem plausible. A heritable basis is further in agreement with the significant genetic differentiation between males from the two populations. However, random effects between geographically separated populations could cause such differentiation at polymorphic loci as well, particularly in a species exhibiting a strongly inbred demic population structure (Selander 1970; Berry 1981). This could potentially render the co-variation of agonistic phenotype and population genotype coincidental. A functional link between the two findings is, however, supported by significant genetic differentiation between agonistic and amicable males of population G. At least, this could suggest possible heritable variation for an agonistic phenotype linked to those markers.

As outlined in the Introduction, the ultimate causation of agonistic phenotype variation between populations might stem from genetic differentiation due to divergent dispersal regimes in feral house mouse populations, as agonistic onset predicts dispersal propensity (Rusu and Krackow, in press), which has been found to exhibit heritable variation in another population (Krackow 2003), and aggressivity measures differ between wild populations exhibiting divergent dispersal regimes (Corti and Rohlf 2001). This conjecture of a dispersal-genotype polymorphism causing agonistic phenotype variability is corroborated by direct molecular evidence in other mammals: In Rhesus macaques (*Macaca mulatta*) a microsatellite polymorphism marks a deletion within the promoter of the serotonin transporter protein gene which starkly reduces dispersal latencies in a natural environment (Trefilov et al. 2000). On the other hand, serotonin is a key component of mammalian aggression regulation (Nelson and Chiavegatto 2001), potentially linking gene expression levels to aggression-mediated dispersive phenotype differentiation. However, a plethora of physiological mechanisms is certainly imaginable, including mechanisms based on parental rather than filial expression of genes.

Theoretically, stable genetic differentiation of dispersal thresholds can be anticipated to occur only under certain conditions (Olivieri et al. 1995). Details of environmental fluctuation, locally co-adapting traits, and demographic characteristics exert significant influence on optimal dispersal propensity and stability of any polymorphism (Kisdi 2002). As such parameters are hard to obtain for house mouse metapopulations, predictions as to the stability of polymorphisms in house mice appear impossible. However, a dispersive morph has also been identified in other rodents (O'Riain et al. 1996), indicating potential genetic control of ontogenetic determination of phenotypes. The empirical evidence, therefore, indicates that further research into the determination of agonistic onset in house mice could be a highly effective means of identifying the genetic basis of dispersal propensity. This could serve as a promising model for studying selection on behavioural syndromes in mammals in general.

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