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Mendelian inheritance of *t* haplotypes in house mouse (*Mus musculus domesticus*) field populations[1]

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Summary

Alleles of many genes in the house mouse (*Mus musculus domesticus*) *t* complex influence embryonic development, male transmission ratio, male fertility and other traits. Homozygous *t* lethal alleles cause prenatal lethality, whereas male *t* semilethal homozygotes and males heterozygous for two complementing *t* lethal haplotypes are sterile. Without a mechanism maintaining these deleterious genes, *t* lethals and *t* semilethals should be eliminated by selection. The mechanism for maintaining them is transmission ratio distortion (TRD), which is said to occur when a *t*/+ male sires a significantly greater proportion of fetuses carrying his *t* haplotype (80–100%) than his wild-type chromosome 17. To understand how this selfish DNA functions in trapped populations, the objectives of this study were to examine the structure of *t* haplotypes in Colorado field populations and to determine transmission ratios in these populations. The data presented here indicate two possible causes for lower than expected transmission ratios in field populations: (1) single-sire fertilization by sperm from mosaic *t* males may lack all *t* haplotype genes causing high TRD. (2) *t*-bearing sperm fertilizing multiple-sire litters are diluted by + sperm from males having the most common genotype (+/+).

1. Introduction

The house mouse *t* complex, 20 cM and 40 Mb adjacent to the centromere on chromosome 17, contains genes influencing the evolution of selfish DNA, including transmission ratio, mate choice, urinary volatiles, male aggression, male fertility and prenatal lethality (Silver, 1985; Ardlie, 1995, 1998; Baker, 1990; Lenington *et al*., 1996; van Boven & Weissing, 1996, 1999, 2001; Durand *et al*., 1997; Erhart *et al*., 2002; Lyon, 2003; Carroll *et al*., 2004; Samant *et al*., 2005). *t* complex-specific probes and primers (Erhart *et al*., 1989; Schimenti & Hammer, 1990; Hammer *et al*., 1991; Morita *et al*., 1993; Herrmann *et al*., 1999; Bauer *et al*., 2005, 2007) provide tools to assess the dynamics of selfish DNA, the *t* complex, in field populations, in particular determining transmission ratio distortion (TRD) of *t* haplotypes in litters of trapped pregnant +/+ dams.

[1] Dedicated to the memory of my parents Selma Gottlieb and Morton Joseph Miller.

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(i) *TRD*

TRD, the most complex and critical component of *t* haplotypes, is said to occur when a *t*/+ male sires a significantly greater proportion of fetuses carrying his *t* haplotype than his wild-type chromosome 17. TRD was assumed to maintain deleterious *t* lethal and *t* semilethal genes in populations (e.g. Lewontin & Dunn, 1960), although more recent models showed that high TRD was unnecessary for the maintenance of deleterious *t* genes (van Boven & Weissing, 1996, 1999, 2001). A formal genetic model for TRD (Lyon, 2003) has included six or more distorters (*Tcd*) acting *in trans* and one responder (*Tcr*) acting *in cis*. Other unspecified loci within and outside the *t* complex appear to influence TRD (Gummere *et al*., 1986; Lyon, 2003). Four inversions in chromosome 17, namely In(17)1, In(17)2, In(17)3 and In(17)4, reduce recombination between *t* and + chromosomes and keep the *Tcd* and *Tcr* genes together, although some recombination occurs (Bennett, 1975; Artzt *et al*., 1985; Ardlie, 1995; Uehara *et al*., 1999; Dod *et al*., 2003; Lyon, 2003). A part of *Tcd-1* (In(17)1), of *Tcd-2*

(In(17)4), and of *Tcr*, between In(17)2 and In(17)3, have been mapped and sequenced (Herrmann *et al*., 1999; Bauer *et al*., 2005, 2007), whereas the remaining unsequenced distorters have only been localized to inversions: In(17)2: *Tcd-4*; In(17)3: *Tcd-3*; and In(17)4: *Tcd-5* (Lyon, 2003).

Genetic changes may decrease transmission ratios. As distorters act additively upon the responder, the absence of a distorter or the responder decreases transmission ratios (Lyon, 2003). Different genetic backgrounds reduce transmission ratios (Gummere *et al*., 1986), although transmission ratios are invariable on some genetic backgrounds (Ardlie & Silver, 1996). The loss of modifier genes over generations may decrease TRD in laboratory lines (Gummere *et al*., 1986), although no genetic modifiers of transmission ratios were found in trapped populations (Ardlie & Silver, 1996).

Sexual behaviour may influence transmission ratios. Whether fertilization during postpartum oestrus decreases transmission ratios is controversial (Lenington & Heisler, 1991; Ardlie & Silver, 1996). Compared with sexual partners from different populations, those from the same population have lower transmission ratios (respectively $81 \pm 6\%$, $N = 19$ *vs*. $54 \pm 6\%$, $N = 15$; Lenington, 1986). If +/+ females mate randomly, they will mate primarily with +/+ males, which represent the most abundant genotype (*c*. 80–90%). Therefore multiple-sire litters will have *t* sperm competing for fertilization not only against their meiotic partners, but against + sperm that have not been modified by *t* proteins during spermatogenesis or during epididymal transit (Olds-Clarke & Peitz, 1985; Ardlie, 1995).

Low transmission ratios (17%) occurred for 3 of 10 trapped pregnant +/+ dams with *t*/+ fetuses (Ardlie & Silver, 1996). Combining these ten trapped pregnant +/+ dams gives a 71.6% transmission ratio $\{[(7 \times 0.95) + (3 \times 0.17)]/10$ with a 95% confidence interval of 38–91%$\}$. When t^{w5}/+ and t^{w1}/+ males mated with females trapped on Eday Island, their F_1 progeny had a 67% estimated combined TRD from 5 litters (no data on individual litters were available; Ardlie, 1995). In contrast, when a trapped pregnant *t*/+ dam mated with an unknown sire, the transmission ratio was 45% for 7 litters with a 95% confidence interval of 13–77% (Ardlie & Silver, 1996).

(ii) *Males with mosaic* t *complex genotypes may have reduced TRD*

A house mouse with a mosaic *t* complex genotype has +/+ and *t*/+ genotypes at different loci (Erhart *et al*., 1989). Mosaicism results from gene conversion (Hammer *et al*., 1991) or recombination (Bennett, 1975; Artzt *et al*., 1985; Dod *et al*., 2003; Lyon, 2003). The proportion of mice with mosaic *t* complex genotypes ranges from 0 to 45.3% depending on genetic marker, geographic area and *t* complex inversion (Silver *et al*., 1987; Figueroa *et al*., 1988; Erhart *et al*., 1989; Hammer *et al*., 1991; Ardlie, 1995; Lyon, 2003; Ben-Shlomo *et al*., 2007). *Tcd* loci and the *Tcr* locus, which cause high transmission ratios when all are interacting, are spread across the *t* complex. Mosaic *t* mice may have no *Tcr* or fewer *Tcd*, resulting in reduced transmission ratios. However, some models show that *t* haplotypes with low transmission ratios may successfully compete against *t* haplotypes with higher transmission ratios (van Boven & Weissing, 1996, 1999, 2001).

(iii) *Objectives*

Mosaicism at the *t* complex could cause low TRD resulting in the possible loss of deleterious *t* haplotypes from field populations. The first objective of the present study was to determine the proportion of trapped Colorado house mice with mosaic *t* complex genotypes. TRD is considered essential for the maintenance of deleterious *t* haplotypes. The second objective was to assess TRD in trapped pregnant +/+ mice with *t*/+ fetuses from Colorado farms.

The data presented here indicate two possible causes for lower than expected TRD in field populations: (1) single-sire fertilization by sperm from mosaic *t* males may lack all *t* haplotype genes causing high TRD. (2) *t*-bearing sperm fertilizing multiple-sire litters are diluted by + sperm from males having the most common genotype (+/+).

2. Materials and methods

(i) *Trapping*

In 1989, 1990, 1995 and 1996, house mice were live-trapped and sacrificed for DNA in 33 Larimer County, Colorado farms or homes and in Senegal (two homes in Dakar; Université de Saint-Louis (Appendix 4, http://journals.cambridge.org/grh)). Shawn Meagher (Western Illinois University) donated one trapped pregnant dam from each of two Florida farms.

(ii) *DNA purification*

DNA for restriction fragment length polymorphisms (RFLPs) was purified by standard methods (Sambrook *et al*., 1989). DNA for PCR was purified by several methods (http://www.jax.org/imr/tail_nonorg.html; Truett *et al*., 2000; Qiagen DNeasy; Gerard Biotech).

(iii) t *complex hybridization probes and primers*

DNA hybridization probes and primers distinguishing *t*/+ and +/+ laboratory strains of trapped mice

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were used: *D17Leh89* (Hammer *et al*., 1991) and *Hba-ps4* (Schimenti & Hammer, 1990) are in In(17)4; Tcp1 (Morita *et al*., 1993), *Bb40* (a part of *D17Leh66*; Ardlie, 1995) and *D17Leh119* (Hammer *et al*., 1991) are in In(17)2. TSE, in In(17)4, was developed to distinguish between *t* haplotypes using two-dimensional electrophoresis (Uehara *et al*., 1999). However, none of these probes or primers distinguished among *t* haplotypes. Standard methods were used to produce probes, and to visualize RFLP on Southern blots.

(iv) t *complex PCR*

Most mice were screened with two *t*-specific primer pairs: *Hba-ps4* (+ band, 198 bp; *t* band, 214 bp; Schimenti & Hammer, 1990; Hammer & Silver, 1993; Huang *et al*., 2001; Dod *et al*., 2003) and Tcp-1 [Morita *et al*., 1993: *t*/+ (1.6 kb) mice have a B2 repeat (200 bp) missing in +/+ mice (1.4 kb); Dod *et al*., 2003]. One of 491 Taiwanese mice (*Mus musculus castaneus*) was +/+ for *Hba-ps4* and *t*/+ for *Bb40*. When *Hba-ps4* and Tcp-1 were screened with DNA hybridization probes, a few exceptions to their *t* specificity occurred (Erhart *et al*., 1989; Hammer *et al*., 1991).

The *Hba-ps4* primers amplified reliably using published protocols (Schimenti & Hammer, 1990), whereas Tcp-1 primers (Morita *et al*., 1993) amplified sporadically. Most of the Tcp-1 genotypes were from primers designed by J.A. DeWoody (Purdue University; + band, 425 bp; *t* band, 600 bp). The sequence of Tcp1jad F is GAC AAT CAT AGC CTT GTC TCA G, whereas the sequence of the Tcp1jad R is GCA GTG TTA TCT TTC ACT GG. PCR reaction conditions for the Tcp1jad primers included: 1.5 mM $MgCl_2$ final concentration; 53 °C annealing temperature; and 35 cycles. Tcp-1 primers designed by C. Landel (Jefferson Medical University) were also used with the + band 517 bp and *t* band 692 bp. The sequence of Tcp1cl F is CTA TGT GGG GCT TGA TTT TCT GTC, whereas the sequence of Tcp1cl R is TGC AAC ATG CTT CAG GTC TCG. Reaction conditions for Tcp1cl included first a Pst-1 digestion for *c*. 4 h at 37 °C followed by PCR with 1.5 mM $MgCl_2$ final concentration; 55 °C annealing temperature; and 35 cycles.

(v) *Multiple-sire litters assessed by microsatellite loci*

In estimating TRD in trapped pregnant +/+ dams with *t*/+ fetuses, it is important to distinguish between single-sire ($\leqslant$2 paternal alleles) and multiple-sire ($\geqslant$3 paternal alleles) litters because TRD is defined for single-sire litters and there is no simple way to distinguish which fetuses are sired by each free-living male. If random mating for a second sire occurs, when one sire is *t*/+, the other will likely be +/+, the most common *t* complex genotype. Two sires is a minimum estimate for a multiple-sire litter because close relatives share alleles, such as brothers or a father and his son.

Multiple paternity analyses require accurate genotype frequencies. To be used in screening trapped mice, microsatellite loci had to have: (1) genotypes reliably scored double-blind; (2) >6 alleles of nearly equal frequency; (3) Mendelian inheritance; and (4) $\leqslant$5% null alleles at each locus in each population (Cervus 2.0; Marshall *et al*., 1998). A high null allele frequency causes mis-scoring of true null allele heterozygotes as homozygotes. Microsatellite primers *D2Mit30*, *D2Mit285*, *D10Mit15*, *D10Mit20*, *D13Mit15*, *D18Mit60* and *D6Mit138* were screened in mice trapped on four Larimer County, Colorado farms: 2M, CM, SL and WO.

Litter size and microsatellite genotype data of $\geqslant$20 adult males and females per farm per locus were used to estimate the probability of detecting multiple-sire litters in a population ($1-Q$; Akin *et al*., 1984). With <20 males, the sampling error makes $1-Q$ an inaccurate predictor. To determine the number of loci for a 90–95% probability of detecting multiple-sire litters, all Q values for different loci for a farm were multiplied and the product subtracted from 100%.

3. Results

(i) *Most of the mice examined had the same* t *complex genotype at different loci*

Trapped mice with mosaic *t* complex genotypes (+/+ at one locus and *t*/+ at another locus) may lack *Tcd* or *Tcr* genes, thus causing a reduction in transmission ratios of *t* haplotypes. A mosaic *t* mouse was classified as *t*/+ (following Ben-Shlomo *et al*., 2007). For mosaicism estimates, all males, nonpregnant females, and only one individual per family (pregnant dam and fetuses) were counted. Of 473 trapped mice screened for $\geqslant$2 loci, only 11% (8.4–14.2%, 95% confidence interval) had mosaic *t* complex genotypes (Table 1). In contrast, 44% of 121 *t*/+ mice (35–53%, 95% confidence interval) screened for $\geqslant$2 loci had mosaic *t* complex genotypes.

Of 53 mosaic *t* mice, nearly four times more mice ($N=42$) were *t*/+ for *Hba-ps4* and +/+ for other loci than the reverse ($N=11$ mice +/+ for *Hba-ps4* and *t*/+ for other loci). Of the seven discordant mice screened for $\geqslant$3 loci, most had one predominant genotype, such as one mouse *t*/+ at 4 screened loci and +/+ at 1 screened locus. Twelve mice (8 mosaic t and 4 concordant; http://journals.cambridge.org/EDE) were excluded from Table 1 because each had a unique genotype combination.

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Table 1. *Mice with the same genotype for all* t *complex loci* (*concordant*) *versus mice with mosaic* t *complex genotypes* (*discordant*). *Complete names for loci are in Materials and Methods. Loci are in the largest inversions*

Inv(17)2			Inv(17)4				
119	Tcp1	40	Hba	TSE	89	Agree	No. mice
t/+		*t*/+	*t*/+	*t*/+	*t*/+	concord	1
+/+		+/+	+/+	+/+	+/+	concord	4
+/+	+/+	+/+		+/+	+/+	concord	2
	+/+	+/+	+/+	+/+	+/+	concord	4
t/+	*t*/+	*t*/+	*t*/+		*t*/+	concord	1
+/+	+/+	+/+	+/+		+/+	concord	16
+/+		+/+	+/+		+/+	concord	2
+/+	+/+	+/+	+/+			concord	3
t/+	*t*/+		*t*/+			concord	1
	+/+	+/+	+/+			concord	2
+/+	+/+		+/+			concord	8
	tt		*tt*			concord	1
	t/+		*t*/+			concord	64
	+/+		+/+			concord	289
+/+			+/+			concord	4
+/+	+/+	+/+	+/+	*t*/+		discord	1
t/+	*t*/+	*t*/+	*t*/+		+/+	discord	1
t/+	+/+	*t*/+	*t*/+		*t*/+	discord	1
t/+		*t*/+	*t*/+		+/+	discord	1
	+/+	*t*/+	*t*/+			discord	1
+/+	*t*/+		+/+			discord	1
+/+	*t*/+		*t*/+			discord	1
	+/+		*t*/+			discord	37
	t/+		+/+			discord	8
t/+			+/+			discord	1

(ii) *Most trapped pregnant +/+ dams had lower than expected TRD*

Previous experiments demonstrated that environmental and genetic variates can change *t* haplotype transmission ratios (reviewed in Ardlie & Silver, 1996), implying that *t* haplotype transmission ratios of free-living *t*/+ males may be more variable than those of caged *t*/+ males. To reduce variance in TRD, workers try to get each caged *t*/+ male to sire *c*. 100 fetuses, which includes several litters (Ardlie and Silver, 1996). As there is no simple and accurate way to determine whether individual males sire more than one field-conceived litter, the number of fetuses in one litter (*c*. 1–15 fetuses) causes a large variance in TRD of free-living *t*/+ males. If high rates of TRD, which are found in most mice breeding in the laboratory, occurred in the 39 trapped pregnant +/+ dams with *t*/+ fetuses, most litters should have 80–100 % *t*/+ fetuses.

Of 224 trapped pregnant +/+ dams, 185 had only +/+ fetuses, whereas 39 had a mean of 49.9 % *t*/+ fetuses (5.5 % standard error; all litters weighted equally; Fig. 1; Appendix 1 in http://journals.cambridge.org/EDE). Nearly the same results occurred when weighting by litter size (total *t*/+ fetuses/total fetuses): the weighted mean is 48.2 % *t*/+ fetuses (5.6 % standard error; http://www.minitab.com.au/support/macros/default.aspx?action=code&id=97). Of the 39 +/+ dams with *t*/+ fetuses, 33 dams were +/+ for ⩾2 loci, whereas 6 dams were +/+ for 1 locus. 12 litters had <20 % *t*/+ fetuses, whereas 9 litters had 100 % *t*/+ fetuses (Fig. 1), which included 5 litters with fetuses having mosaic *t* genotypes and 9 litters having *t*/+ fetuses screened for ⩾2 loci. 10 litters had only fetuses with the same genotype at different loci. 13 litters had more than one-third fetuses with mosaic *t* complex genotypes. Of 211 fetuses

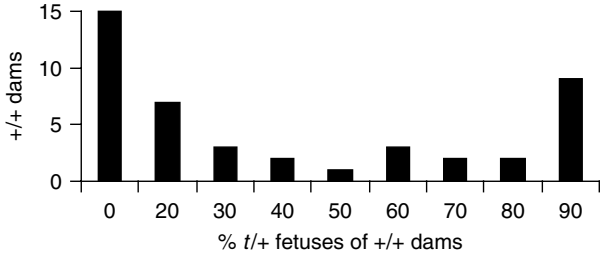

Fig. 1. A total of 39 trapped +/+ pregnant dams were sacrificed to obtain their fetuses. The mean *t* haplotype transmission ratio was 49.9 % with a standard error of 5.5 %. 30 of these 39 +/+ dams had fetuses with mosaic *t* complex genotypes. Following Ben-Shlomo *et al*. (2007), a mosaic *t* mouse was classified as *t*/+.

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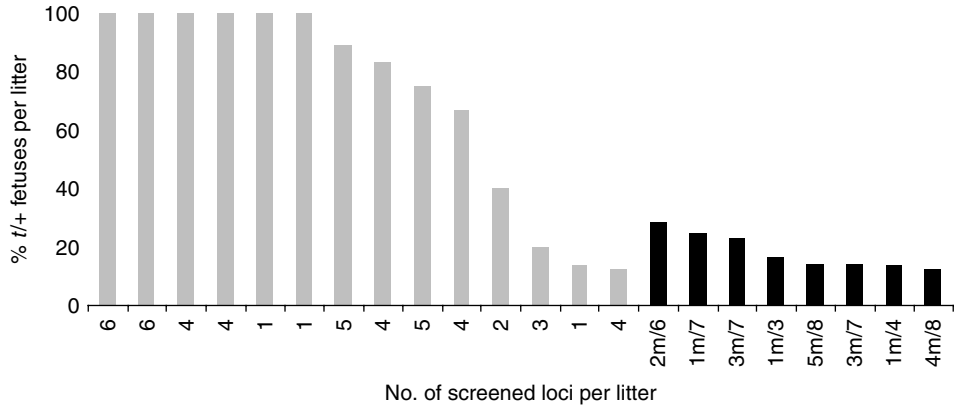

Fig. 2. Single-sire litters ($\leqslant 2$ paternal alleles; grey bars) have a significantly higher proportion of $t/+$ fetuses than multiple-sire litters ($\geqslant 3$ paternal alleles; black bars; Appendix 2, http://journals.cambridge.org/grh). The number of microsatellite loci screened is below each litter; for multiple-sire litters, the numerator is the number of loci with $\geqslant 3$ paternal alleles, whereas the denominator is the total loci screened.

screened for $\geqslant 2$ loci, 56 had mosaic t genotypes (26%).

(iii) *Single-sire litters have higher proportions of* t/+ *fetuses*

In free-living populations, a litter could be sired by multiple males (Dean *et al.*, 2006). As shown in Fig. 2, multiple-sire litters had significantly fewer $t/+$ fetuses (18.5 ± 2.2% standard error) than single-sire litters (71.5 ± 9.3% standard error; Mann–Whitney $U = 95$; $N_1 = 14$ single-sire litters, $N_2 = 8$ multiple-sire litters; $P < 0.01$, two-tailed test; http://elegans.swmed.edu/~leon/stats/utest.cgi). If a dam mates randomly, the second sire will probably be $+/+$, because this is the most common t complex genotype, *c.* 80–90% $+/+$ mice.

The probability of discriminating single versus multiple-sire litters increases with the number of loci screened. To have a 95% probability of detecting multiple-sire litters on four farms (2M, CM, SL and WO), 4 loci (*D2Mit30*, *D10Mit15*, *D13Mit15* and *D18Mit60*) were screened for 20 males per farm; the other screened loci included *D2MIt285*, *D6Mit138* and *D10Mit20*. Litters with $\leqslant 3$ screened loci failed to amplify or were from pregnant mice trapped in less intensively genetically screened farm populations. The less intensive screening of other farm populations means that the true multiple-sire litters from these farms may be classified as single-sire litters.

(iv) t/+ *dams have more* t/+ *fetuses than mosaic* t *dams*

Understanding t haplotype dynamics in field populations includes determining the proportion of $t/+$ fetuses of $t/+$ trapped dams. Captive $t/+$ females have about equal numbers of $+/+$ and $t/+$ fetuses (Ardlie & Silver, 1996; Carroll *et al.*, 2004). The mean

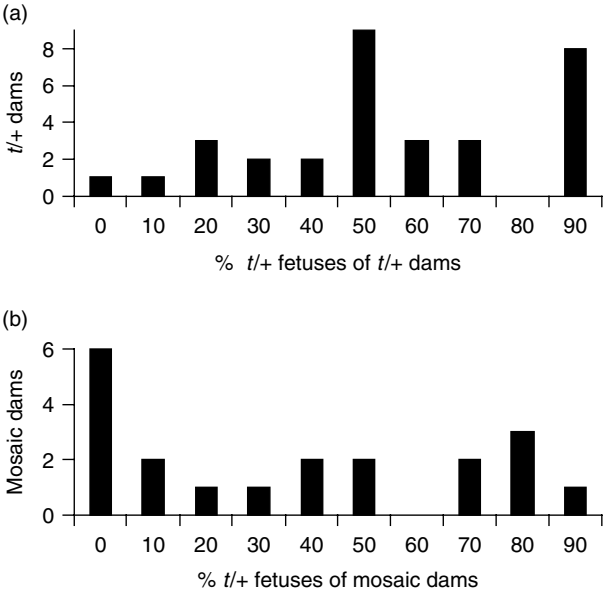

Fig. 3. (*a*, *b*) A total of 32 $t/+$ dams and 20 t mosaic dams had significant differences in percentage of $t/+$ fetuses.

proportion of $t/+$ fetuses of 32 $t/+$ dams was 59.3% (5.2% standard error) when all litters were weighted equally, and nearly the same (56.6%; standard error 4.8%) when weighted by litter size. The mean proportion of $t/+$ fetuses of 20 mosaic t dams was 38.8% (7.7% standard error) when all litters were weighed equally, and nearly the same (35.9%; 7.5% standard error) when weighted by litter size. Six mosaic t dams had no $t/+$ fetuses, which contributed to their lower percentage of $t/+$ fetuses.

Thirty-two $t/+$ trapped pregnant dams had significantly more $t/+$ fetuses than 20 mosaic t trapped pregnant dams (Fig. 3; Mann–Whitney $U = 434.5$, $N_1 = 32$, $N_2 = 20$; $P = 0.03$; http://elegans.swmed.edu/~leon/stats/utest.cgi). $t/+$ dams had a bimodal distribution at 50–60 and 90–100% $t/+$ fetuses, whereas

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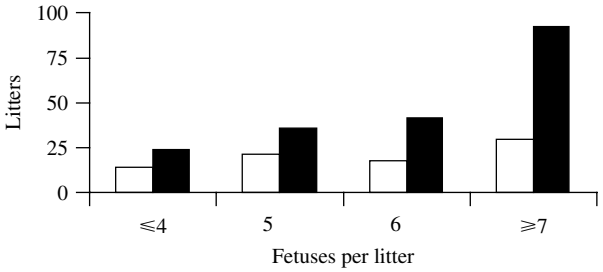

Fig. 4. +/+ dams (black bars) have nearly significantly larger litters than *t*/+ dams (white bars). Litter size ranged from 1 to 15 fetuses.

mosaic *t* dams had a unimodal distribution at 0% *t*/+ fetuses.

If a mosaic *t* dam is +/+ at a locus for which its fetus is *t*/+, a *t*/+ male sired that fetus. This reasoning may underestimate the number of fetuses sired by the *t*/+ male because his+sperm may fertilize eggs. *t*/+ males sired fetuses of seven mosaic *t* dams as follows: two *t*/+ fetuses of nine screened littermates, abbreviated as 2*t*/9, 5*t*/7, 3*t*/7, 4*t*/5, 3*t*/4, 2*t*/4 and 2*t*/3.

(v) t/+ *dams have smaller litters than* +/+ *dams*

Captive studies showed that *t*/+ parents had smaller litters than +/+ parents (Dunn & Suckling, 1955; Dunn *et al.*, 1958; Johnston & Brown, 1969; Levine *et al.*, 1980; Lenington *et al.*, 1994; Johnson *et al.*, 1995; Ardlie & Silver, 1996; Carroll *et al.*, 2004). Fecundity data from trapped +/+ and *t*/+ dams (Fig. 4) tend to support this conclusion (Mann–Whitney $U=9239$; $N_1=194$ litters of +/+ dams, $N_2=83$ litters of *t*/+ and mosaic *t* dams; $P=0.051$, two-tailed test; http://elegans.swmed.edu/~leon/stats/utest.cgi), underscoring the potential effect of differential fecundity as another cause for low *t* haplotype frequencies in field populations.

(vi) *Few* t/t *mice occurred*

Lewontin & Dunn (1960) emphasized the importance of high transmission ratios in maintaining deleterious *t* haplotypes in populations, implying fetal *t* lethal homozygotes are common. *t* complex genotypes for each farm in Fig. 5 were from all males, nonpregnant females, and one representative (usually pregnant dam) from each family (dam and her fetuses). These data showing low proportions of *t*/+ mice. If mating occurs randomly, most *t*/+ mice will mate with +/+ mice, which will keep rare the deleterious *t* phenotypes expressed by *t*/*t* mice (prenatal lethals and male sterility).

Of 2480 mice genetically screened, only 6 *t*/*t* mice were found, which is 0.20% of the total screened: 1 *t*/*t* fetus of a *t*/+ pregnant dam (89sj39) trapped in Larimer County, Colorado; 4 *t*/*t* fetuses of a *t*/+ pregnant dam trapped in Dakar, Senegal (96da6)

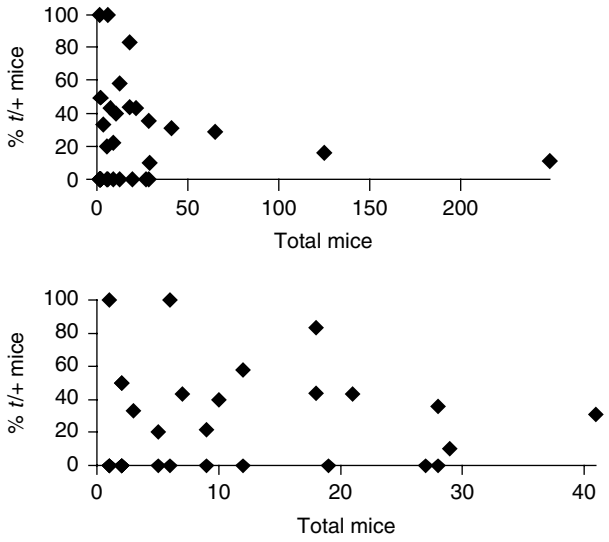

Fig. 5. Large populations may have lower *t*/+ frequencies. The upper figure includes all farms (Appendix 3, http://journals.cambridge.org/grh), whereas the lower figure includes ⩽41 mice per farm.

and 1 *t*/*t* adult trapped in Pakistan (FB10350; DNA donated by Kristin G. Ardlie and Francois Bonhomme). Since the 4 *t*/*t* fetuses are from one litter, and thus dependent data, 3 of the 4 Dakar *t*/*t* fetuses were omitted, resulting in a total of 3 *t*/*t* of 2480 mice, which is 0.12% of the total screened.

t/*t* mice include *t* lethal homozygotes, which die prenatally, heterozygotes for two complementing *t* lethal haplotypes and *t* semilethal homozygotes. With the possible exception of t^{w1} homozygotes, which can die between neural tube degeneration and birth, the most common *t* lethal haplotypes in North America cause early prenatal deaths and resorption of *t*/*t* embryos (Bennett, 1975). Therefore resorbing dead *t* lethal homozygous embryos and fetuses will be undetected in sacrificed trapped pregnant dams. Another logistical problem in identifying *t* lethal homozygotes is fetal resorption occurring in litters of *t*/+ dams. Six *t*/+ dams, 8+/+ dams with +/+ fetuses and 2+/+ dams with *t*/+ fetuses had fetuses that were smaller, a different colour (brown, grey *vs.* pink), or had no obvious fetal morphology compared with other fetuses in the litter. Even if these resorbing fetuses could be collected, a mixture of maternal and fetal membranes surrounds these resorbing fetuses. To mitigate potential maternal DNA contamination of fetal DNA, large fetuses were removed from their surrounding membranes, whereas small fetuses were omitted from TRD estimations.

4. Discussion

(i) *Conclusions*

This study documented three causes of decreasing *t* haplotype frequencies: (1) mosaic *t* males may lack

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the *Tcr* and *Tcd* genes causing high transmission ratios; (2) in multiple-sire litters, the proportion of *t*-bearing sperm fertilizing eggs is reduced by +-bearing sperm that are not directly affected by *t* haplotype proteins as are the +-bearing sperm that were meiotic partners of *t*-bearing spermatids; (3) *t*/+ parents have fewer pups than +/+ parents. This study documents one cause of increasing *t* haplotype frequencies: nine trapped +/+ dams had only *t*/+ fetuses; i.e. 100% TRD.

More (11%) North American mosaic *t* mice were found in this study than the one putative mosaic *t* mouse trapped in Michigan (Erhart *et al*., 1989; contradicted by Hammer *et al*., 1991). Previously, most mosaic *t* mice had been trapped in Europe and the Middle East (Figueroa *et al*., 1988; Erhart *et al*., 1989). *Tcr* and *Tcd* loci act additively to cause the highest ratios of transmission distortion (Lyon, 2003). If some *Tcr* or *Tcd* loci are missing in mosaic *t* mice, then lower transmission ratios will occur. A low transmission ratio occurred for the single-sire litter of dam 952M99, which was screened for four loci, sufficient to detect with a 95% probability a multiple-sire litter. Other causes for low TRD of trapped litters include genetic modifiers of distortion; mutations in distorter loci; *t* haplotype variability; individual variability; short duration between insemination and fertilization; and genetic backgrounds on the same and on the homologous chromosome 17 (reviewed in Ardlie & Silver, 1996).

A total of 39 trapped pregnant +/+ dams had a mean of 49.9% *t*/+ fetuses, which is lower than that reported in laboratory mating (90–100%) or in 10 trapped +/+ pregnant mice with *t*/+ fetuses (71.6%; Ardlie & Silver, 1996).

(ii) *Maintenance of deleterious* t *haplotypes in field populations*

High TRD was assumed to maintain deleterious *t* haplotypes in field populations. If low TRD is widespread in field populations, how can deleterious *t* haplotypes be maintained? Two causes for the maintenance of deleterious *t* haplotypes were documented in the present paper and a third cause was deduced from published studies:

(1) High TRD occurring in some trapped pregnant mice (9 of 39 litters, the present paper; 7 of 10 litters, Ardlie & Silver, 1996) will help maintain deleterious *t* haplotypes.
(2) Low TRD occurring in *t*/+ and mosaic *t* parents will help maintain deleterious *t* haplotypes as follows. Low *t* haplotype frequencies (*c*. 10–20%) are common in most free-living populations (Ardlie & Silver, 1998). If random mating occurs, then most deleterious *t* haplotypes will be carried by *t*/+ mice. Deleterious phenotypes, such as prenatal lethality and male sterility, are expressed by *t*/*t* mice, which are rare.

Of 3263 trapped and genotyped mice, Ardlie & Silver (1998) trapped 27 *t*/*t* (0.82%) mice from four farms in New Jersey and Iowa, whereas Dod *et al*. (2003) trapped 1 *t*/*t* mouse of 1068 mice from 135 Danish buildings (0.09%). Significant differences occur in the proportions of *t*/*t* mice across populations [A. E. M. Baker's 0.12% (3 of 2480 mice) *vs*. Ardlie and Silver's (1998) 0.82% *vs*. Dod *et al*.'s (2003) 0.09%]; significant differences at the 95% confidence level occurred, with the exception of Ardlie and Silver (1998) (0.82%) *vs*. Dod *et al*. (2003) (0.09%; http://survey.pearsonncs.com/significant-calc.htm). Causes for these significant differences include chance sampling predominating in small isolated house mouse populations and differences among sampled *t* haplotypes.
(3) Population size influences selection against deleterious *t* haplotypes and genetic drift causing random changes in deleterious *t* haplotype frequencies. In large populations, selection and multiple-sire litters (Dean *et al*., 2006) predominate in causing the elimination of deleterious *t* haplotypes. However, in small populations, chance events and single-sire litters predominate, which can increase deleterious *t* haplotype frequencies.

(iii) *What next?*

The influence of mosaic *t* mice on TRD is an unresolved controversy (Erhart *et al*., 1989, 2002; Hammer *et al*., 1991; van Boven & Weissing, 1996, 1999, 2001; Dod *et al*., 2003). Resolving this controversy requires: (1) screening mosaic *t* males for all *Tcd* and *Tcr* loci to determine whether mosaic *t* males have sufficient genes to cause high transmission ratios; and (2) trapping pregnant +/+ dams with *t*/+ fetuses and screening their fetuses for all *Tcd* and *Tcr* loci to determine whether transmission ratio varies directly with the expression and number of *Tcd* and *Tcr* loci. Studies of mate choice and breeding by mosaic *t* males could determine whether mosaic *t* males have the same reproductive fitness as +/+ males, but have a higher reproductive fitness than nonmosaic ('complete t') *t*/+ males.

Studying *t* haplotype dynamics over generations in populations with competing *t* haplotypes (Bennett, 1975) will provide insights into the population genetics of the *t* complex. House mice live in many ecological situations, which could be studied to determine the influence of ecological factors on transmission ratios. For example, population density has a rough positive correlation with the number of multiple-sire litters (Dean *et al*., 2006) and a rough

negative correlation with the proportion of males (Baker, 1981).

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