Multiple paternity and differential male breeding success in wild ship rats (*Rattus rattus*)

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Abstract: Multiple paternity increases the genetic diversity of litters, hence could have two important implications for the control of invasive pests in which multiple paternity is common. (1) Migrating pregnant females could establish a new population with substantial genetic variation from the first generation; (2) Existing populations could recover from a control operation with minimal bottleneck effect. We therefore sought information on the extent of this character in ship rats (*Rattus rattus*), and on the probability of pregnant females avoiding capture or moving to new areas. We genotyped the embryos carried by 17 pregnant female ship rats collected from eight forest fragments trapped to extinction in rural Waikato, North Island, New Zealand. Best results were obtained from a northern subgroup of five forest fragments, all located within 5 km of each other, where we had data for 57 candidate fathers, and 71 embryos in 15 litters. We matched 12 fathers with 24 embryos (34% of offspring) through correspondence of two independent analytical methods, using detailed ecological data to add to the value of the paternity data and exclude false matches. Six of the 12 northern-group fathers had contributed to only one litter each, whereas three were represented in three litters each and three in two litters. Additional fathers were identified by one or other method alone. A further 45 sexually mature males were present, but they were not definitely linked to any of the 71 northern embryos sampled, even though the genotypes of both were known. Another 55 embryos were genotyped but not firmly matched to any father. Multiple paternities were the norm: only one complete litter could be attributed to a single father, and all others had between two and four fathers. Only nine of the 101 old females (>121 g) caught at any time were marked with Rhodamine B dye, available only outside the trapping areas, whereas 18 of 62 old males (>141 g) were marked, of which 14 were caught after 7 days of trapping. We conclude that multiple paternity benefits surviving resident females of breeding age, whereas many breeding males are very mobile and benefit by moving on soon after mating.

Keywords: bottlenecks; control of invasive pests; founder effects; mating behaviour; sperm competition

Introduction

Control and management of common pest species subject to high natural mortality rates are of wide general interest (Caughley 1977). One example, the ship rat (*Rattus rattus*), is a small mammal of tropical origin now globally distributed due to an impressive record of island invasions, and to its legendary resistance to subsequent risks of local extinction (Stolzenburg 2011). Ship rats are widespread and abundant on the New Zealand mainland and offshore islands (Towns & Broome 2003; Towns et al. 2006), with serious conservation consequences (reviewed by Innes (2005)). They disperse very widely and breed promiscuously. Females will accept several males during a given fertile period, so the resulting set of embryos may have more than one father (Miller et al. 2010). We investigated the potential role of multiple paternity in explaining this remarkable capability for establishing new populations and persisting in spite of bottlenecks.

The frequency of multiple paternity in the wild has real significance for studies both of new invasions and of recovery of managed populations of ship rats. Multiple paternity plays a significant role in maintaining genetic diversity by increasing the effective population size (Sugg & Chesser 1994). If multiple paternity is common, models of invasions should allow for its influence in minimising the genetic bottleneck effect normally associated with founder events (Abdelkrim et al. 2005; Russell et al. 2009), and increasing the survival chances of a new colony derived from a single pregnant invader (Miller et al. 2010). Multiple paternity in rats is easily detectable by standard genetic techniques, but it requires separate sampling of all the embryos in a set and their mothers. To identify the fathers with a high probability, all the mature males must also be sampled. For any large, widely scattered and mobile wild population, this is a lot of work, and it has never been done for ship rats. Hence, very little is known about the extent of multiple paternity among wild ship rats, its potential consequences for the reproductive success of adult rats of different ages, and its implications for our understanding of the invasive dynamics of this widespread pest.

In a pioneering study of multiple paternity, Miller et al. (2010) genotyped three litters of wild ship rats. Neither the genotypes of the potential fathers, nor the ages or status (resident in a home range or not) of the mothers, were known, so paternal alleles were inferred by eliminating known maternal alleles, assuming no allelic dropout or null alleles. On these criteria, two sets of embryos were each inferred to have been fathered

by at least two males, while the third set detected only a single set of male alleles (Miller et al. 2010).

This paper describes part of a wider study of key ecological processes on pastoral land around rural Waikato, New Zealand. Elsewhere we have described the abundance of ship rats in eight forest fragments (Innes et al. 2010), the eradication of all eight populations (n = 517 rats caught), the inevitable reinvasion, and a second eradication, all between January and May 2008 (King et al. 2011). We investigated the population structure and genetic diversity of the rats originally present, and the significant difference in age structure and reproductive activity between them and the rats reinvading the cleared areas. The 366 rats of both sexes and all trappable ages caught during the first eradication, including 32 pregnant females, are the subjects of this paper.

We took advantage of the unusual opportunity presented by our previous work to obtain information about multiple paternity and variance in breeding success in wild ship rats. The existing database contained the genotypes of all the mothers, and, we hypothesised, presumably also those of at least some of the potential fathers, of the 32 litters we collected. We aimed to: (1) sample the embryos and match their genotypes with those of their mothers, and document the number of litters fathered by more than one male; (2) where possible identify the fathers; and (3) estimate the minimum extent of differential breeding success among males from the number of litters to which each contributed.

Methods

Study areas

The eight fragments studied are scattered along a range of hills 20–30 km south-east of Hamilton City in the central Waikato region, North Island, as mapped in King et al. (2011) and described by Innes et al. (2010). All the fragments represent cutover remnants of previously continuous podocarp–broadleaved evergreen native forest invaded by ship rats after about AD 1860 (Atkinson 1973). The northernmost five fragments (numbered 1, 2, 4, 7 and 8) were all within 5 km of each other and at least 12.5 km from the southernmost three (numbered 3, 5 and 6), which were all within 2 km of each other. The eight areas averaged 5.3 ha in size (range 2.4 to 9.9 ha).

As it was logistically impossible to treat all the fragments simultaneously, the eradication in Fragments 1–4 was started earlier (10 January 2008) than in Fragments 5–8 (14 February), which meant that many more pregnant rats came from Fragments 1–4 than from 5–8. This did not affect our main conclusion that all rats in all eight fragments belonged to a single metapopulation (King et al. 2011). Gene flow and individual movements between the sampled populations and the source areas surrounding them made our analysis more difficult than comparable studies on islands, but more typical of mainland rat infestations.

Rat population data

We divided the collection into three age-related groups by sexual maturity and body weight, separately for each sex. The definition of age by weight was significantly correlated with tooth-wear categories and with the different proportions of reproductive activity among weight groups of both sexes (King et al. 2011).

The oestrous cycle in the female is 4–6 days long, and the gestation period 20–22 days. We collected 32 pregnant rats classed in the 'old' age category (>121 g) , all trapped in January or February, most within 6 days of the first clearing of the traps on 11 January; other old females were still lactating well into March (King et al. 2011). The number of pregnant females we recorded was necessarily underestimated by up to a third, because the uterine swellings containing the early embryos are not visible to the naked eye during necropsy for about their first week (Innes et al. 2001). The number of embryonic genotypes available for analysis was further reduced because the embryos inside the smallest of the visible swellings could not be accurately sampled without risk of contamination with maternal tissue.

Implantation of ova fertilised during a post-partum oestrus may be delayed by lactation (Innes 2005). Hence, the span between the known capture dates for potential parents did not need to be limited to 22 days. Mantalenakis and Ketchel (1966) determined that, in Norway rats, delayed implantation after fertilisation post-partum resulted in gestation periods averaging 26 days. In the absence of any other information, we assumed the same applied to ship rats. We used this period to determine the maximum prior date of mating for a pregnancy that had not yet reached term. Hence, when matching potential fathers to embryos, we ruled out any males removed more than 26 days before the capture date of the mother. We refer to this window as our '26-day rule'.

We used the methods fully described by King et al. (2011) to distinguish between resident and other rats. We placed a vital dye, Rhodamine B (RhB), in bait stations in adjacent areas outside our study areas. The whiskers of rats eating dyed baits became marked with fluorescent bands. Marking implies that a rat trapped in a fragment is likely to be a recent immigrant (see the original analysis for reasoning).

From each captured rat, we removed a 20-mm piece of ear tissue, using sterile scissors, and stored it in a tube containing 95% ethanol for subsequent DNA analysis. From each female containing visible embryos, we excised the uterus and stored it complete in 70% ethanol in a labelled ziplock bag, and subsequently frozen at − 18°C. In the laboratory, we opened the uteri, removed the individual amniotic sacs from each embryo, and excised tissue from the embryo for digestion. Closer examination found that 15 uteri contained embryos too small to sample, and five of the remaining 17 sets included at least one embryo smaller than the others in its set, presumably resorbing.

DNA extraction and genotyping

We extracted genomic DNA both from ear tissues and embryos, using the Corbett X-tractor Gene™ automated DNA solid-tissue extraction system (Corbett Robotics, Brisbane, Australia) following the manufacturer's recommendation, with external digest. We used nine microsatellite markers, previously developed from *R. norvegicus* genome mapping (Jacob et al. 1995), to genotype each sample: D2Rat234, D5Rat83, D7Rat13, D10Rat20, D11Mgh5, D15Rat77, D16Rat81, D18Rat96, D19Mit2. We performed PCR amplification in 10-µl volumes, following Abdelkrim et al. (2010), and ran the PCR products as two multiplex runs on an ABI prism 3130 capillary electrophoresis system (Applied Biosystems). We derived genotypes using GeneMapper ® v 4.1 software (Invitrogen Life Technologies). From the tissue genotypes, we calculated an overall P_{sib} score using GenAlEx v 6.41 (Peakall & Smouse 2006) in order to determine the probability that a sibling would possess an identical genotype. For further details of the population genotypes, see King et al. (2011).

Paternity matching

We replicated DNA extraction, PCR and genotyping for each embryo, with a consensus score taken for each marker. Since, with simple inheritance, one allele for each locus should be inherited from the mother, the genotypes of a mother and each of her litter can be compared. If neither allele at a locus matched those of the known mother at that locus (a mismatch), the mother's tissue was re-extracted, and PCR, genotyping and scoring repeated to reduce the likelihood of any error. We did the same wherever null alleles were revealed in the mother's genotype, appearing as mismatches between mother and offspring's genotypes necessarily involving homozygotes. The existence of a prior database (King et al. 2011), documenting the allele distributions in all captured rats from the sampled populations, greatly increased the probability of our achieving correct results.

We searched for potential fathers from among all the known reproductively mature males (i.e. excluding only juveniles). To permit sufficient numbers of candidate fathers to be analysed, we pooled data from the group of five northern fragments, since our previous study (King et al. 2011) had showed no apparent genetic differentiation within this group. We likewise pooled data for the three southern fragments.

For the northern group of five fragments, and the southern group of three fragments, we calculated allele frequencies for the whole population collected from each group, of all ages and sexes. Candidate fathers were restricted to those reproductively mature males in each dataset that could have been alive at the same time as the mothers, i.e. up to the end of March. Wherever the data offered a choice between assigned fathers, we accepted the one that matched with the highest probability, and where both were equally probable, we accepted the one that had been caught at the trap closest to the mother. In either case we recorded both, but have reported the less probable matches separately.

Analysis and modelling

We used the software packages NewPatXL (Worthington Wilmer et al. 1999) and CERVUS 3.0 (Kalinowski et al. 2007) to analyse paternity. Each uses slightly different statistics and assumptions.

NewPatXL is an exclusion software program that identifies matches according to user-determined levels of null alleles and mismatches for mothers, offspring, and candidate fathers, factoring in the size and number of repeat units in scoring. The strict exclusion approach retains validity even if levels of relatedness in a population are extremely high, e.g. half siblings from prior litters (Jones & Ardren 2003). We applied it using strict exclusion criteria, permitting no paternal mismatches, a low level (0.05) of acceptable null allele matches in mothers or fathers, and no unscored loci. Each paternity assignment is given a probability that the data would show such a match by chance by generating 'pseudomale genotypes' from random alleles. Through setting this to 100 times the size of the candidate male dataset, these randomisation numbers equate to a percentage probability that the match would be generated from these data by chance alone. This method avoids any need to predict what proportion of candidate fathers was sampled, which CERVUS requires.

CERVUS is a categorical allocation software program that finds which of the candidate fathers is likeliest to be the true father for each offspring, determined by the delta (log-likelihood) score. Given the known genotype of the mother, the likelihood of each paternity match can be assigned confidence intervals at relaxed (80%) or strict (95%) levels as appropriate. We simulated the paternity matching in CERVUS to determine whether it would be possible to identify matches with any reliability. Because our grouping of fragments effectively incorporated unsampled habitats between fragments to which true fathers might have moved, we assumed we had sampled only 50% of all possible candidate fathers. Using the real data for allele frequencies, simulation parameters were 10 000 offspring, the proportion of loci typed = 0.993, mistyping error rates = 0.001 (northern) / 0.0152 (southern), and likelihood calculation error rates = 0.001, permitting 1 unscored locus.

We calculated the straight-line distances in metres between the trap sites where identified fathers and mothers were caught, and the intervals between their trapping dates. We discarded any proposed paternity assignments that exceeded our '26-day rule'.

Results

Exclusion probabilities for each marker are reported in Table 1, along with summary statistics (Table 2), but are not used as a measure of confidence, as they assume an absence of mutations and scoring errors (Jones et al. 2010). Given that some markers departed from Hardy–Weinberg equilibrium, we analysed data excluding these markers for comparison. We present data with the markers included, as the non-exclusion probabilities were higher with fewer loci, thus less conservative in finding matches. Simulation results using CERVUS are shown in Table 3, giving paternity matches predicted and observed for the dataset.

Paternity analysis by NewPatXL and CERVUS

NewPatXL could identify no matches as fathers for any embryos from five of the 15 litters (33%) genotyped in the northern group, and one of two in the southern (Table 4). NewPatXL (Table 5a, b, d) found 31 offspring matches with 12 fathers from among the 57 candidate males in the northern group dataset. After we excluded two NewPatXL matches between rats with capture dates separated by >26 days (011, 076; Table 5d), 29 of 71 (41%) northern embryos could be attributed to 10 fathers among 57 candidates. Only one complete litter could be attributed to a single father, 042, in the northern group. For the southern group, one embryo (11%) was assigned to one father from the 27 candidates.

CERVUS identified paternity assignments for 31 embryos at the strict (95%) confidence level (Table 3, 5a, c, d) from among northern fragments under the 50% candidate father sampling criterion. After two CERVUS paternity matches were excluded under the 26-day rule (025, 076; Table 5d), 41% of the embryos were assigned, involving 10 fathers. A further 19 (cumulative 70%) were assigned at the relaxed level, involving 22 fathers. These percentage assignments are respectively lower and higher than the 51% and 57% suggested by the simulation (Table 3) indicating that our assumption as to the proportion of candidate fathers sampled was reasonable.

In the southern group, CERVUS strict level support was identified for two assignments (22%) (Table 3) and relaxed support for a further three (cumulative 56%, not listed), half of the 44% and slightly greater than the 50% indicated by the simulation. The assumption of 50% candidate father sampling is thus also probably reasonable among the southern fragments, given the lower numbers.

Table 1. Microsatellite data summary for ship rats (*Rattus rattus*) collected in Waikato, North Island, New Zealand, between January and April 2008. Abbreviations: k = no. of allelic states; Hobs = observed heterozygosity; Hexp = expected heterozygosity; PIC = polymorphic information content; NE-2P = non-exclusion probability of second parent with first parent known; HW = significance of deviation from Hardy-Weinberg equilibrium (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS not significant); F(Null) = estimated null allele frequency.

	Locus	D2	D5	D7	D11	D19	D10	D15	D16	D18
Northern	k	16	10	11	17	11	9	13	8	14
	Hobs	0.777	0.716	0.647	0.727	0.900	0.260	0.811	0.524	0.782
	Hexp	0.786	0.788	0.735	0.784	0.832	0.382	0.812	0.561	0.871
	PIC	0.757	0.758	0.694	0.762	0.810	0.359	0.786	0.500	0.856
	NE-2P	0.403	0.406	0.491	0.390	0.333	0.785	0.365	0.692	0.261
	HW	NS	NS	NS	**	*	***	NS	NS	***
	F(Null)	0.0051	0.0490	0.0617	0.0427	−0.0412	0.1970	−0.0017	0.0257	0.0539
Southern	k	9	9	11	9	12	7	9	4	14
	Hobs	0.694	0.701	0.765	0.479	0.965	0.265	0.769	0.555	0.890
	Hexp	0.725	0.772	0.782	0.53	0.797	0.370	0.756	0.516	0.854
	PIC	0.679	0.732	0.751	0.507	0.766	0.351	0.713	0.443	0.835
	NE-2P	0.509	0.449	0.414	0.661	0.396	0.790	0.471	0.743	0.292
	HW	NS	NS	NS	NS	***	***	NS	NS	NS
	F(Null)	0.0174	0.0470	0.0058	0.0524	−0.1072	0.1897	−0.0083	−0.0402	−0.0236

Table 2. Summary statistics.

	Northern group	Southern group
Number of individuals	292	150
Number of loci	9	9
Mean number of alleles per locus	12.11	9.33
Mean proportion of individuals typed	0.9935	0.9637
Mean expected heterozygosity	0.7278	0.678
Mean polymorphic information content (PIC)	0.6982	0.6418
Combined non-exclusion probability (first parent)	0.01135	0.02896
Combined non-exclusion probability (second parent)	0.00054	0.002
Combined non-exclusion probability (parent pair)	2.7E-06	2.6E-05
Combined non-exclusion probability (identity)	3.77E-10	7.13E-09
Combined non-exclusion probability (sibling identity)	0.00031	0.00064

Table 3. CERVUS critical log likelihood scores, number of paternities predicted (generated from 10 000 simulated offspring) and observed for this dataset, taking account of the mother's known genotype. Candidate father sampling modelled at 50%.

Population	Confidence level	Critical ΔLOD score	Predicted paternities (expected)	Paternities allocated (observed)
Northern group	Strict: 95%	2.31	36 (51%)	31 (44%)
	Relaxed: 80%	0.00	41 (57%)	50 (70%)
Southern group	Strict: 95%	1.77	4 (44%)	2 (22%)
	Relaxed: 80%	0.00	5 (50%)	5 (56%)

Paternity assignment comparison

After exclusions, of the total 29 northern group paternities identified by NewPatXL and 29 at strict level by CERVUS, 24 assignments were shared, representing 80% commonality. Of the assignments identified by NewPatXL, but not given 95% CI in CERVUS (Table 5b), four were nevertheless supported by CERVUS at 80% CI. One was not, and also had an inter-trapping interval of 26 days, suggesting it might be spurious (063, Table 5b). Embryo 017-4 was allocated to two separate fathers by NewPatXL. The first match, to father 027 was not supported by CERVUS, the second, to father 030, was supported at 80% only. Of the five strict-level assignments found by CERVUS, but not by NewPatXL (Tables 3, 5c, d), each contained a single mismatch with the father's genotype, not permitted by the NewPatXL analysis. Three were also assigned to the same father as a litter-mate, thus making each assignment more plausible, while the remaining two assignments were to fathers not identified by NewPatXL.

In the southern group, CERVUS included one additional strict and three relaxed assignments. The additional strict assignment contained a mismatch with the paternal genotype, while the relaxed assignments also included at least one mismatch. All assignments made by NewPatXL were also recovered by CERVUS.

After 26-day exclusions, all the matches in the northern group identified fathers that had been caught in the same fragment as the mother. For the 24 assignments in common between methods, the mean inter-parent trapping distance per litter was 157 m (± 68 m SD). Including the additional assignments by each NewPatXL and CERVUS, this distance rises to 186 m (± 70 m).

Four of the nine northern-group fathers recovered by both methods had contributed to only one litter each after exclusions (or six of 12 including assignments without common support; Table 4a). Conversely, three fathers were represented in two litters each and three in three litters (including assignments without common support). Another 45 sexually mature males

Table 4. Summary of paternal assignments for embryos of *Rattus rattus* from Waikato, North Island, New Zealand, and comparison between methods.

Mother ID	Litter size	NewPatXL No. embryos assigned to fathers	Matches excluded by 26-day rule	No. fathers	CERVUS No. embryos assigned to fathers 95% CI	Matches excluded by 26-day rule	No. fathers	80% CI
Northern								
002	7	5		4	6		4	7
004	5	5		1	5		1	5
010	2	0		0	0		0	0
017	5	4		3[1]	2		1	4
022	4	3		2	3		2	3
052	5	3		2	2		1	5
065	4	0		0	0		0	2
090	5	0		0	1		1	2
100	6	2		2	2		2	4
101	6	2		2	3		2	4
108	6	2		1	2		1	4
137	2	2	1	1[2]	0		0	2
193	4	2		1	3		2	4
196	5	0		0	1	1	0	2
223	5	1	1	0	1	1	0	2
	71	**31** 43.7%			**31** 43.7%			**50** 70.4%
Southern								
033	6			1			2	3
054	3			0			0	2
	9	**1** 11.1%			**2** 22.2%			**5** 55.6%

[1] Embryo 017-4 assigned with equal probability to two fathers.
[2] Embryo 137-1 assigned with equal probability to two fathers, of which one father excluded by 26-day rule.

were present, but they were not definitely linked to any of the 71 embryos sampled even though the genotypes of both were known. For Fragments 2 and 4, the locations of both the confirmed fathers and the genotyped males not known to be fathers are shown in Figs 1 and 2.

For two of the three southern Fragments, trapping started late (14 February), and no pregnant females were caught there. In the only other southern fragment, trapped in January, we sampled two litters (n = 9 embryos), and screened 27 candidate fathers collected from all three fragments, of which one was assigned paternity of one of six embryos. Hence only approximately 11% of embryos could be attributed to a father from the southern dataset, and no fathers could be identified for the three embryos in the other litter (Table 4). While multiple paternity in this area was also indicated by the offspring alleles in relation to the mothers, the evidence was weaker. The distance between the capture locations of the one matched father and mother was 102 m.

The two fragments with the highest abundance of rats and the most reproductive activity were both fenced blocks in the northern group sampled in January. Fragment 2 was connected to a source of immigrants 210 m away by a narrow corridor, and Fragment 4 was isolated by at least 250 m of open pasture. Presenting our results specifically by location and date, including additional data from King et al. (2011) on locations of trap sites, hair-tubes, and ages and distributions of marked and non-breeding animals, identified biologically implausible matches and adds an intriguing spatial element to the picture. The combined data suggest considerable variance in breeding success among the confirmed fathers (Figs 1 & 2).

Discussion

One of the most puzzling aspects of our results is that, although our large database of potential fathers included every mature male collected from each fragment, there were still 21 individual northern embryos, including one whole litter, for which no father(s) could be identified at any level of significance. Similarly, there were 4 of 9 southern embryos unassigned, even at 80% significance. We can suggest two reasons for this.

(1) The RhB data showed that rats were frequently moving into and out of the fragments even before the eradications began, especially after the first week of trapping to extinction (King et al. 2011). It is therefore probable that the father(s) responsible for the unattributed embryos left the trapping area before being caught. Conversely, adult males whose genotypes were known but could not be linked to an embryo may have arrived after fathering litters elsewhere, or have contributed to any of the 15 recorded litters containing only embryos too small to genotype.

(2) The criteria for declaring each eradication complete were severe (Innes et al. 2010), so we think that the alternative explanation (that some fathers remained in the area but were not caught during the sampling period) is less likely.

Table 5. Capture details for male *Rattus rattus* from Waikato, North Island, New Zealand, identified by NewPatXL and CERVUS as assigned fathers of genotyped embryos collected in the northern group of five fragments. (a) Assigned by correspondence of both methods; (b) additional NewPatXL assignments shown italicised: (c) additional CERVUS strict assignments shown in bold type; (d) additional assignments discarded under 26-day rule. Asterisks identify males identified by one method but otherwise unknown. Calculations of inter-parental trapping distances shown separately for the nine cases where both methods agreed, and the total of 12 cases. Three further cases excluded by the 26-day rule are listed but not included in distance estimates. All but two assignments are recorded from Fragments 2 and 4, as plotted in Figs 1 and 2.

Male ID	Fragment	Body mass (g)	Date trapped (dd/m/yy)	No. trapped litters to which this male contributed	Embryos assigned	Distance (m) from father's to mother's trap site	Date mother trapped (dd/m/yy)	No. inter-trapping days (−ve: mother before father)	Comments
(a) Assigned by both methods									
019	2	146.8	11/1/08	1	002-1	120	13/1/08	2	
027	2	194.6	11/1/08	3	002-4	0	13/1/08	2	
					022-1	91	11/1/08	0	
030	2	191.4	11/1/08	2	022-2	162	11/1/08	0	
					022-4			0	
042	2	174.0	12/1/08	3	002-3	29	13/1/08	1	
					002-7				
					004-1	98	13/1/08	1	Mother RhB +ve
					004-2				
					004-3				
					004-4				
					004-5				
					017-3	47	11/1/08	−1	
					017-5				
063	4	143.7	16/1/08	2	108-3	102	29/1/08	13	
066	4	178.3	17/1/08	2	100-6	93	27/1/08	10	
					101-2	148	27/1/08	10	
067	4	186.4	17/1/08	3	052-1	61	14/1/08	–3	
					052-2				
					101-2	170	27/1/08	10	
					101-6				
085	2	138.9	24/1/08	1	002-6	0	13/1/08	−11	
278	8	198.2	15/2/08	1	193-1	35	15/2/08	0	
					193-2				
Total 9 recovered by both methods				Litters per father (mean ± SD) 1.44 ± 0.73	Embryos per father (mean ± SD) 2.56 ± 2.60	Distance (m) per litter (mean ± SD) 157 ± 68			
(b) Additional assignments by NewPatXL (*otherwise unknown)									
027	2	194.6	11/1/08	3	*017-4*	24	11/1/08	0	Also assigned to 030
030	2	191.4	11/1/08	2	*017-4*	257	11/1/08	0	Also assigned to 027
063	4	143.7	16/1/08	2	*108-2*	102	29/1/08	13	
					137-1	72	11/2/08	26	
067	4	186.4	17/1/08	3	*100-3*	114	27/1/08	10	
109*	4	193.7	30/1/08	1	*052-3*	102	14/1/08	−16	
(c) Additional strict assignments by CERVUS (*otherwise unknown)									
012*	4	205.3	11/1/08	1	**090-1**	73	25/01/08	14	
027	2	194.6	11/1/08	3	**002-2**	0	13/1/08	2	
063	4	143.7	16/1/08	2	**108-6**	102	29/1/08	13	
067	4	186.4	17/1/08	3	**101-1**	170	27/1/08	10	
218*	8	191.5	15/2/08	1	**193-4**	246	15/2/08	0	
Total 12 recovered by all methods				Litters per father (mean ± SD) 1.75 ± 0.87	Embryos per father (mean ± SD) 2.92 ± 2.50	Distance (m) per litter (mean ± SD) 186 ± 70			
(d) Additional assignments discarded under 26-day rule									
011*	4	215.9	11/1/08	1	*137-1*	151	11/2/08	31	011 assigned by NewPatXL
025*	2	152.8	11/1/08	1	**196-5**	2,208	21/2/08	41	025 assigned by strict CERVUS
076*	2	187.1	20/1/08	1	223-4	2,146	27/2/08	38	Assigned by both methods

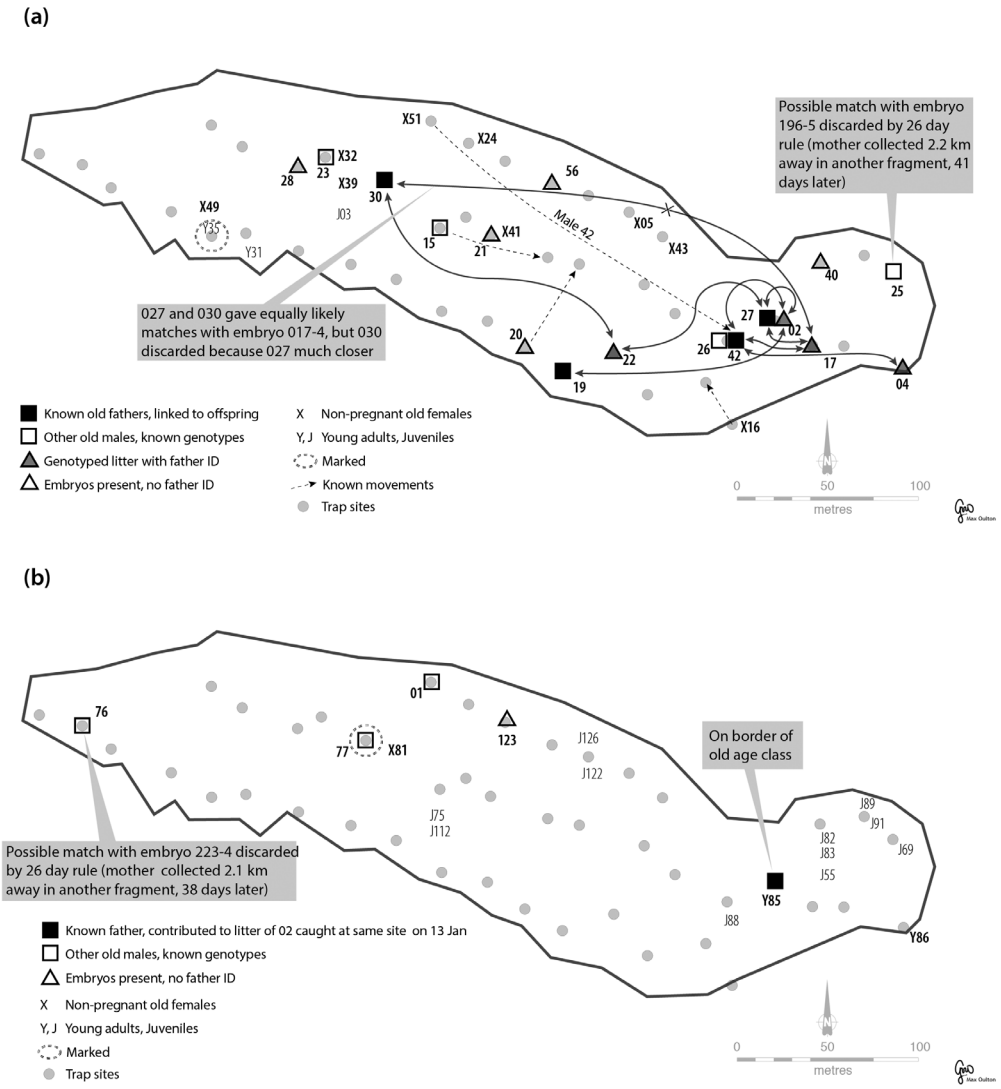

Figure 1. Capture and hair-tube locations (described in King et al. 2011) identifying spatial distributions of ship rats (*Rattus rattus*) collected from Fragment 2, Waikato, North Island, New Zealand. (a) Rats collected on the first 6 days, 11–16 January 2008, and (b) after 6 days, on 17 January to 16 February 2008. Curved solid arrows link identified fathers with the capture location of the female carrying the litter to which each father contributed (links marked with an X show paternity records identified by genetics but rejected on ecological grounds; see text). Straight dashed arrows link sites of hair-tubes and traps at which the same individual was recorded. Sexually mature adults not identified as parents, and young and juveniles of both sexes, are also shown. Individuals marked with RhB dye are ringed. For further details, see Table 5.

Conversely, our modelling showed that the probability of an assigned father not being the true father was only 0.15% under the strict condition in the CERVUS simulation, provided the true father was among the candidate fathers we sampled. This result gives us confidence that the paternal assignments listed in Table 5 are correct.

Multiple paternity

In the five northern fragments, multiple paternities within a litter were definitely the norm: only one of 17 sampled litters could be attributed with confidence to a single father, 042 (Tables 4 & 5a). In the three southern fragments, multiple paternity was also indicated by the distribution of offspring alleles in relation to the mothers, although we found only one corresponding match to a single embryo with NewPatXL/CERVUS and one more strict assignment with CERVUS alone. Of the total 17 litters genotyped, we identified between two and four fathers for five litters (29%) with CERVUS and for six litters with NewPatXL (35%) (Table 4).

Such a promiscuous mating system implies extensive sperm competition, which in turn is often correlated with a large testis volume relative to body weight and/or with relatively large sperm (Breed & Taylor 2000). In a survey of 100 species of murine rodents, *R. rattus* was placed in the highest category for both (Breed & Taylor 2000). Sperm competition was inferred by Dean et al. (2006) to be high in wild house mice (*Mus domesticus*) in which multiple paternity was detected in 33 of 143 litters of at least three embryos (23%). Intensive sperm competition implies high levels of virility, another reason for the global success of invasive pests such as commensal rats and mice.

Variation in male breeding success

In this study we were able to take advantage of an unusual opportunity to document variance in reproductive success among adult male rats in a wild population. In species with intense sexual selection and promiscuous mating, skewed paternal success rates are to be expected (Breed & Taylor 2000), but for rats it is very difficult to demonstrate them in the wild. While our data suggest that some males were indeed more successful than others (probably the largest, oldest and most experienced competitors), the reverse is not necessarily true, because we could not sample all embryos collected, and for three litters we could not identify any fathers (Table 4).

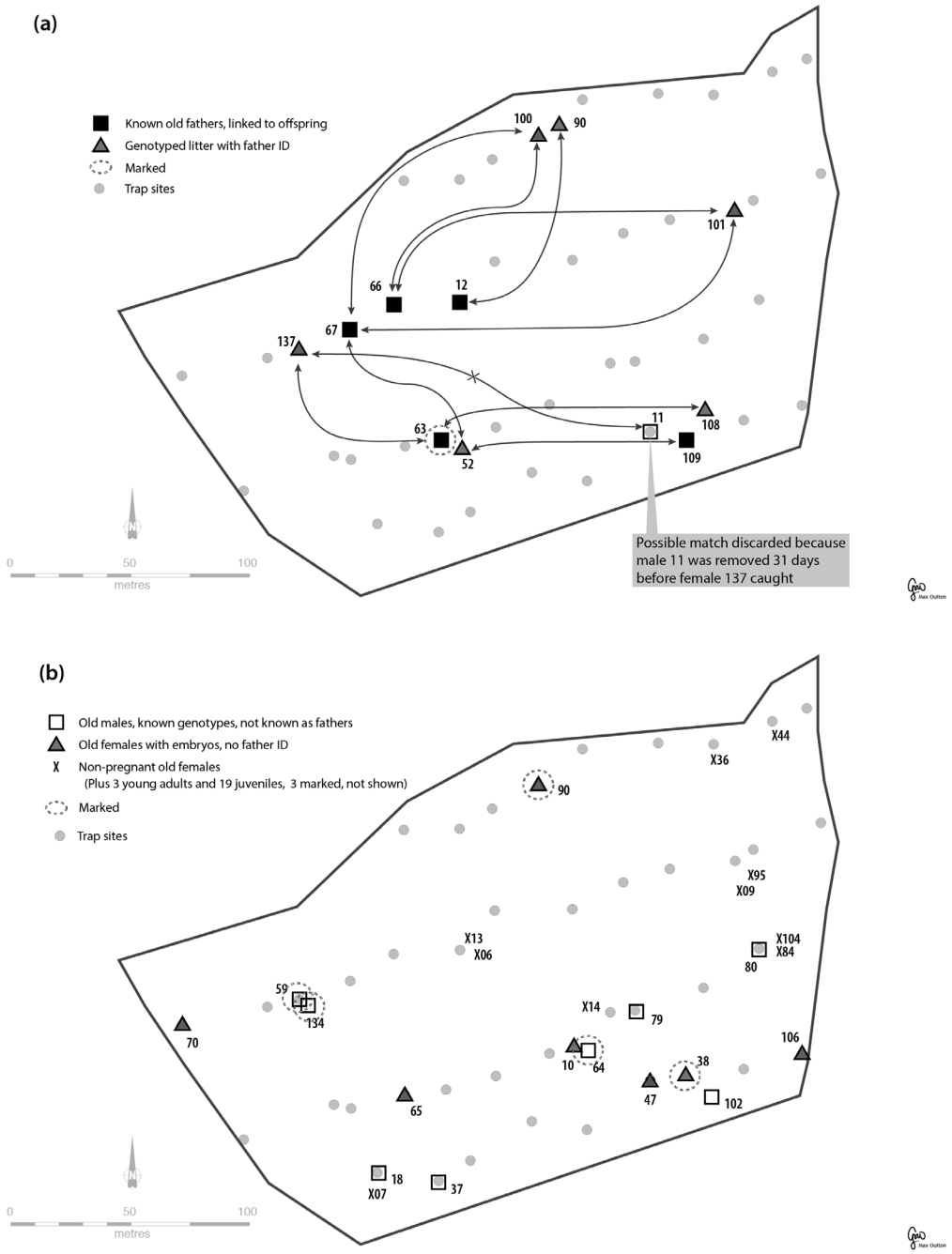

Figure 2. Capture locations for ship rats (*Rattus rattus*) collected from Fragment 4, Waikato, North Island, New Zealand. (a) Known fathers and their offspring collected from 11 January to 17 February, and (b) all other rats caught over the same period. Arrows link identified fathers with the capture location of the female carrying the litter to which each father contributed (links marked with an X show paternity records identified by genetics but rejected on ecological grounds; see text). Sexually mature adults not identified as parents, and young and juveniles of both sexes, are also shown. Individuals marked with RhB dye are ringed. For further details, see Table 5.

For three further litters (Table 5d), the paternity assignments made were discarded under our 26-day rule.

Our results are consistent with several possible reasons for individual variation in mating success observed in *R. norvegicus,* but which have never been tested in *R. rattus*. Zewail-Foote et al. (2009) placed together 11 trios of two sexually experienced male rats plus one naïve female, and found that, among the eight litters produced, one of the two males had sired most, if not all of the pups. Lovell et al. (2007) demonstrated a significant effect of female choice in captive Norway rats, and Russell et al. (2009) estimated that breeding success among individuals belonging to one small island population of Norway rats was dominated by one male and one female. Johnson and Gemmell (2012) reviewed the apparent paradox that, although sperm function generally declines with age in many species, including captive Norway rats, female mammals often prefer older males.

We consider the most likely explanation for the differences we observed is that the populations we sampled were not closed. We are reasonably sure that no resident rats survived the eradications, but we could not detect links between parents of either sex that had fathered or conceived litters outside our study areas. We suspect that breeding females are more sedentary than breeding males because, among the 74 marked rats in this collection (caught during the first eradication), only nine were old females, two of them pregnant, but 18 were old males. We do not know how many successful fathers arrived or left our study areas before trapping began, but our data suggest that breeding males frequently move about in search of mates because, from the seventh day of trapping onwards,

14 of 31 old males (45%) were marked, compared with 4 of 31 (13%) caught on the first six days. Both sets of figures point to high levels of landscape-scale movement and genetic homogeneity between these forest fragments, as we suggested previously (King et al. 2011) from analysis of data without inclusion of embryos.

These data are consistent with previous observations suggesting extensive male mate-searching behaviour. Old adult males are very mobile and hold larger home ranges than old females (Hooker & Innes 1995). Our field data provided a warning that, without further information we cannot a priori rule out the long-distance paternity assignments listed in Table 5d (and others assigned with lower confidence, not listed) as false.

On the other hand, our 26-day rule is based on unconfirmed extrapolation from breeding data on captive Norway rats. If our 26-day rule is incorrect, then the three discarded matches in Table 5d could in fact be valid, including the two linking old male and female rats collected in different fragments. We have no positive grounds to reject their implication that short-term residency and long-distance travel by individual old male rats could be an adaptive strategy increasing individual breeding success. Moreover, males that move on are probably better able to avoid the risk of mating with their own offspring, which can reach sexual maturity within 3–4 months (Innes 2005). New studies exploring this question would need to search for marked rats across much wider areas than has previously been considered necessary.

The RhB data suggest that old females were the most sedentary rats resident within the fragments. All but nine of the 101 rats in this group caught were unmarked, and all of the matched hair and ear samples described by King et al. (2011) came from old females caught on the first day of a trapping session (not all visibly pregnant). Larger, older and socially dominant rats are often the first to explore new devices containing food, and so are most likely to be the first to be caught when traps are newly set. If successfully raising a litter is associated with residency, not with dispersal, and if pregnant rats do not routinely disperse, then perhaps the potential adaptive advantage of multiple paternity is less to do with maximising the diversity of a new population than with re-establishing an existing population after a catastrophic reduction. Conversely, young and juvenile rats dominated the catch after the seventh day of trapping (King et al. 2011), as illustrated in Fragment 2 (Fig. 1b).

Conclusion

We designed this study to investigate the extent and potential consequences of multiple paternity for the control of an invasive pest, the ship rat. We found that multiple paternity was indeed common within the metapopulation we sampled. We hypothesised two possible consequences: (1) multiple paternity could permit migrating pregnant females to establish a new population with substantial genetic variation from the first generation onwards; and/or (2) it could enable existing populations to recover from a control operation with minimal bottleneck effect. Our results identified few invading rats as pregnant females, suggesting that the first consequence is probably less significant than the second.

Russell et al. (2009) commented that the combination of ecological and genetic data can greatly increase the information value of analyses such as these. Our dataset on genetic variation in both adult and embryonic wild ship rats is derived from a very large sample of known genotypes from eight populations trapped to extinction, so is unusual in that it offered the chance to use paternity tests to identify the actual fathers and to check the proposed paternity assignments against field data. Despite this advantage, and the double effort required to replicate the DNA extraction, PCR and genotyping for each embryo, ambiguous results were common, and even strict matches could be ruled out by trapping data. Our experience emphasises the importance of accounting for allelic dropouts and null alleles, and the risks of attempting paternity assignments from genetic data alone, unsupported by supplementary ecological information.

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