

Geographic variation of multiple paternity in the American lobster, *Homarus americanus*

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Abstract

We studied the frequency of multiple paternity for American lobster (*Homarus americanus*) at three Canadian sites differing in exploitation rate and mean adult size. The probability of detecting multiple paternity using four microsatellite loci and 100 eggs per female was in excess of 99% under various scenarios of paternal contribution. Overall, 13% of the 108 examined females carried a clutch sired by two or three males. Multiple paternity was observed at the two most exploited sites (11% at Magdalen Islands and 28% at Grand Manan Island), whereas single paternity only was observed at the least exploited site (Anticosti Island). Within populations females with a clutch sired by more than one male tended to be smaller than females with a clutch sired by a single male. Based on these and other findings, we postulate a link between female promiscuity and sperm limitation in the American lobster.

Keywords: crustacean, marine invertebrates, mating systems, multiple paternity, promiscuity

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Introduction

There is a growing awareness in the scientific literature that hunting and fishing, when practiced in an unrestrained manner, have the potential to perturb the mating system of species (Ginsberg & Milner-Gulland 1994; Orensanz *et al.* 1998). This may be especially true when the largest individuals of one sex are culled more than those of the other sex, and the sex ratio becomes skewed and the least exploited sex has fewer opportunities for mating and/or exercising mate choice (Smith & Jamieson 1991; Chapman *et al.* 2004). The American lobster (*Homarus americanus*, Nephropidae), henceforth simply called the lobster, is exploited throughout its range and supports one of the most important marine fisheries in North America (FRCC 1995). Lobster removals may be biased to males because regulations protect brooding females and fishing seasons often coincide with periods of peak activity and catchability of males (Templeman 1936; Skud & Perkins 1969; Campbell 1992). Heavy exploitation also distorts the lobster size structure and reduces mean individual size relative to

virgin or lightly fished populations (FRCC 1995; Gendron & Gagnon 2001).

Despite more than 60 years of research since Templeman's (1934) influential paper on mating in the lobster, knowledge of the mating system of this territorial species remains incomplete. We know that courtship roles are partially reversed: males compete for territory and defend shelters from which they chemically advertise their presence, while females actively seek mates and compete for access to dominant males and their shelters (Atema *et al.* 1979; Cowan & Atema 1990; Snyder *et al.* 1992; Bushmann & Atema 1997). Accepted females settle into the male's den where they molt and are mated shortly after (Atema *et al.* 1979; Bushmann & Atema 1997). The male passes sperm to the female's single seminal receptacle and inserts a sperm plug that quickly hardens; this was believed to prevent other intromissions until the female's next molt, when the chitinous receptacle and its content are shed (Templeman 1934, 1936). Mating is typically followed by coercive post-copulatory guarding that is usually limited to the brief period after molting during which the female is too soft to challenge or oppose the male (Nelson & Hedgecock 1977; Atema *et al.* 1979). When the female is strong enough to leave the male's den, she may, for sometime, continue a sexual association with the male in the form of intermittent shelter sharing (Atema *et al.* 1979). Females may prefer

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large males because they have larger dens, are more protective and allocate more sperm than smaller males (Gosselin *et al.* 2003). Mated females extrude and fertilize their eggs externally (Aiken *et al.* 2004) usually about 10–12 months after molting (Herrick 1911; Templeman 1934).

Templeman (1934) inferred from observations of captive lobster that both hard-shell (i.e. intermolt) males and soft-shell (i.e. postmolt) females could be promiscuous (here promiscuity is used to describe any mating system in which individuals mate more than once; for mating system terminology, see Shuster & Wade 2003). While male promiscuity was accepted without questioning, female promiscuity was not, perhaps because of prevailing societal prejudices with regard to sex roles (see Birkhead 2000). The discovery by Nelson & Hedgecock (1977) of unexpected allele frequencies at one allozyme locus in the clutch of one wild female later confirmed that female lobster can be polyandrous. Nevertheless, these last two authors predicted that the incidence of multiple mating and paternity would be very low because females were guarded throughout the receptive period which they assumed lasted only a day or so after the molt, as reported by Herrick (1911) and Templeman (1934, 1936). Subsequent laboratory behavioural studies established that females could mate anytime after molting and in the hard-shell condition, although receptivity was greatest during postmolt and pre-oviposition periods (Dunham & Skinner-Jacobs 1978; Waddy & Aiken 1990; Snyder *et al.* 1992). However, microsatellite analyses of some eggs from 13 wild female lobsters from the Gulf of Maine (USA) failed to detect a single case of multiple paternity (Tam & Kornfield 1996).

The very limited genetic investigations to date of paternity patterns for lobster may not adequately portray the incidence of multiple paternity, because the frequency and degree of promiscuity can vary temporally, spatially or phenologically within species (Sainte-Marie *et al.* 2002; Roy 2003). In the specific case of lobster, one might expect the frequency of female promiscuity to be higher in heavily exploited populations than in lightly exploited populations owing to the scarcity of large males. Indeed, laboratory observations indicate that females are more restless (Gosselin *et al.* 2003) and a cursory report suggests they are more inclined to promiscuity (Easton 1976) after mating with a small male compared to a large male. These observations may be related to the lesser amount of sperm passed by small males relative to large males (Gosselin *et al.* 2003). Additionally, one might expect the incidence of promiscuity between molts to change with female size. Indeed, female clutch fecundity is correlated to female body size (Attard & Hudon 1987) and females larger than 120-mm carapace length (CL) typically have intermolt periods of more than 2 years during which they may produce two or more sequential egg clutches (Waddy & Aiken 1990). It follows that female sperm requirements will increase with size.

The objective of this study was to explore the paternity patterns for clutches at three sites in eastern Canada: the Magdalen Islands and Anticosti Island in the Gulf of St. Lawrence, and Grand Manan Island in the Bay of Fundy. The estimated exploitation rate and mean male CL are, respectively, 75% and 90 mm at the Magdalens compared to 20% and 109 mm at Anticosti (FRCC 1995). At Grand Manan Island, we sampled an annually recurrent aggregation of very large males (mean CL of 142 mm during our sampling period) and berried females (Campbell 1990). The estimated exploitation rate in lobster fishing area 38, which includes Grand Manan Island, may range from 53% to 70% (see DFO 2001). However, the large males and females that aggregate at Grand Manan Island may migrate from offshore areas where exploitation rates are much less and it is yet unclear where these large females are mated (see Campbell 1986, 1990; Robichaud & Campbell 1995).

Materials and methods

Collection of females and study sites

Sampling techniques and effort varied among populations because of logistic reasons. Fishermen and Department of Fisheries and Oceans (DFO) personnel collected females brooding ripe eggs at the Magdalen Islands (MI, 47°23'N, 61°48'W) in June 2000 and at Anticosti Island (AI, 49°18'N, 62°00'W) in July 2000 and shipped them by plane or truck to the Maurice Lamontagne Institute. Divers collected females brooding ripe eggs in the shallow waters (1–20 m) of Flag Cove, adjacent to the port of North Head, Grand Manan Island (GMI, 44°45'N, 66°45'W) in July 2000 (see collection methods in Lawton *et al.* 2001a, b). The carapace length (CL) of all lobsters was measured to the nearest 0.1 mm using a vernier caliper as described in Attard & Hudon (1987).

DNA extraction, amplification of microsatellites

Analysis of paternity was performed on 36 randomly selected large (> 90 mm CL) females from Grand Manan Island (see Table 1), and on 12 females randomly selected from each of the three size categories (small, 70–79 mm CL; medium, 80–89 mm CL; large, > 90 mm CL, see Table 1) represented at both Anticosti and Magdalen islands. A small segment of one walking leg (pereopod) was taken from each female for genotyping. Ten eggs were randomly selected from each of the female's 10 abdominal swimmerets (pleopods) and the remaining eggs were brushed off and preserved in 100% ethanol. Grand Manan lobsters were released at the site after sampling. Total DNA was isolated from female pereopod muscle tissue, separate pools of eggs from each pleopod, or individual eggs using the

Table 1 Mean carapace length ± 1 SE and median with minimum and maximum values in parentheses (all values are in mm) for each size class of female American lobster (S, small; M, medium; and L, large) and sampling site. MI, Magdalen Islands; AI, Anticosti Island; GMI, Grand Manan Island

Size class	Site		
	MI	AI	GMI
S	75.7 \pm 0.6	76.4 \pm 0.9	—
	75.7 (71.9–78.4)	77.6 (71.5–79.8)	—
M	87.3 \pm 0.8	85.0 \pm 0.7	—
	88.1 (80.5–89.9)	84.8 (81.9–89.6)	—
L	114.4 \pm 7.4	108.1 \pm 4.0	127.5 \pm 3.1
	107.5 (90.3–170.0)	106.9 (90.7–133.9)	124.9 (94.6–166.1)

Table 2 Summary statistics for four microsatellite loci used on American lobster from Magdalen, Anticosti and Grand Manan islands (36 individuals from each site), including sample size (n), number of alleles per locus, annealing temperature (T_a , in °C) and the average exclusion probability

Locus	n	No. of alleles	T_a	Exclusion probability
<i>Ham 6</i>	108	21	60	0.854
<i>Ham 10</i>	108	12	52	0.613
<i>Ham 21</i>	108	14	52	0.634
<i>Ham 44</i>	108	24	52	0.872

Combined exclusion probability for the three populations for all four loci > 0.996 .

extraction protocol and solutions from QIAGEN DNeasy Tissue Kit. Primers for four lobster microsatellite loci (*Ham 6*, *Ham 10*, *Ham 21*, *Ham 44*, from Jones *et al.* 2003) were used. The allele sizes for those loci ranged from 104 bp to 258 bp and the number of alleles was 12–24 (Table 2). Samples were analysed using the fluorescent detection method. For the polymerase chain reaction (PCR), only the forward primer oligos of each microsatellite were labelled with one of three colours: yellow (HEX) for *Ham 10*, blue (FAM) for *Ham 21*, and green (TET) for *Ham 6* and *Ham 44*. All four loci were amplified separately. PCR conditions were optimized using a touchdown protocol on a GeneAmp PCR System 9700 thermocycler (Applied Biosystems).

Amplification of each microsatellite locus was carried out in a 10- μ L reaction volume containing 1 μ L of 10 \times buffer [10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 0.1% Triton X-100, 50 mM KCl], 1 μ L of 40 mM dNTP mixture, 0.1 μ L of 2 units/ μ L *Taq* DNA polymerase, 1 μ L of 10 μ M forward (F) primer, 1 μ L of 10 μ M reverse (R) primer, 4.9 μ L of distilled water and 1 μ L of DNA extract (50–100 ng). The PCR protocol was the following: mixtures heated at 94 °C for 5 min; five cycles of 92 °C for 1 min, $T_a + (5 \text{ }^\circ\text{C}, 4 \text{ }^\circ\text{C}, \dots 1 \text{ }^\circ\text{C})$ for 30 s, 72 °C for 15 s; 25 cycles of 92 °C for 1 min, T_a

for 30 s, 72 °C for 15 s; and 72 °C for 30 min. The annealing temperature (T_a) suitable for each locus is presented in Table 2.

We produced genotype profiles of females and separate pools of eggs from each of their pleopods (i.e. 10 pleopods + 1 pereopod = 11 samples per female). PCR products were combined and mixed with 2 μ L of distilled water, 1.25 μ L of formamide, 0.75 μ L of blue loading buffer and 0.15 μ L of GeneScan-500 ROX internal size standard. A volume of 1 μ L of this mixture was loaded on a horizontal 5% polyacrylamide gel for a 2 h electrophoresis at 3000 V in an automated DNA Genotyper (BaseStation, MJ Research). The fragment sizes were determined by comparison with the internal standard used in each lane using CARTOGRAPHER software (MJ Research).

For each locus and each population, observed heterozygosity, mean number of alleles and conformity to Hardy–Weinberg equilibrium (HWE) (exact test) were analysed from female muscle samples using the program GENEPOP 3.1 (Raymond & Rousset 1995). The Markov chain method was used to estimate probability values for departure from Hardy–Weinberg. Probability values were greater than 0.05 for all loci at all three populations and therefore showed no significant deviations from the null hypothesis of equilibrium. Paternity exclusion probabilities were calculated for each locus, and cumulatively for each population, under the assumption that the mother's genotype was known (Weir 1996). The four loci used in this study did not exhibit null alleles and did not deviate from Mendelian expectations (see also Jones *et al.* 2003).

Paternity analysis

The maternal genotype was determined from female muscle tissue, whereas the paternal genotype(s) was (were) inferred from nonmaternal alleles expressed in the offspring. The method of pooling eggs from individual pleopods, also used with snow crab (*Chionoecetes opilio*), offers the possibility of screening large clutches (Urbani *et al.* 1998). Because 10 eggs were randomly selected from each pleopod and later pooled for extraction, a minimum of one half-sib (an egg sired by a different father) per sample is possible. To confirm that we could detect this half-sib in samples of 10 pooled eggs, we analysed eggs from two allopatric mothers exhibiting single paternity mixed in proportions of 1:9, 2:8, 3:7, 4:6, and 5:5. Sixteen different pairs of females were tested for each of the mixture proportions, and in each of the resulting 80 combinations we were able to detect all of the expected alleles.

The probability of detecting multiple paternity ($PrDM$) was determined from a Monte Carlo simulation incorporating the effects of number of loci, number of alleles, and the reproductive skew of fathers (Neff & Pitcher 2002). In our study, mean $PrDM$ for each population was based on

the four loci, two contributing fathers, and paternal relative contributions of 50:50 or 90:10. A larger number of fathers would only increase the probability of detecting multiple paternity. However, the probability of detecting multiple paternity depends on the number of eggs that are sampled and we ran simulations using 20, 30, 50 or 100 eggs. *Ham 6* and *Ham 44* allele frequencies used in the calculations were based on the female data set for each population.

Multiple paternity was concluded only if more than two nonmaternal alleles occurred in more than one locus in the progeny arrays, to allow for the possibility of mutation at one locus. Progeny arrays meeting this criterion were re-analysed to confirm results, i.e. both amplification and electrophoresis were repeated. Furthermore, for every confirmed case of multiple paternity, a genotype profile was produced for each of 30 randomly selected eggs using *Ham 6* and *Ham 44* to determine the relative contribution of the different fathers.

Two methods for inferring the minimum number of fathers were used. The first was a single-locus manual assignment approach: the minimum number of males contributing to a female's progeny was taken to be half the number of nonmaternal alleles recorded at the locus, rounded to the next upper integer when the quotient was uneven (Urbani *et al.* 1998). This method may underestimate the actual number of fathers because it assumes that males are heterozygotes and that there is no allele sharing among fathers or between mother and father(s) (Urbani *et al.* 1998). The second method was also a conservative estimate of the number of fathers, based on a multilocus approach, that was derived using the GERUD program (Jones 2001). This estimation procedure uses information on the segregation of paternal alleles and deviation from Mendelian expectations, and expected frequencies of genotypes in the population. GERUD was used with data derived from analysis of individual eggs that were genotyped with *Ham 6* and *Ham 44*.

The frequency of multiple paternity in populations may be underestimated by paternal allele counts, for the same reasons the number of fathers may be underestimated (see previous discussion; Neff *et al.* 2002; Toonen 2004). We used the f_{mm} computer program developed by Neff *et al.* (2002) to evaluate the mean and 95% confidence interval of frequency of multiple paternity (f_{mm}) for our three populations. The program is based on a single-sex model that incorporates in a Bayesian framework information on the (i) number of loci, (ii) number of alleles and their frequencies, (iii) number of broods, (iv) number of eggs analysed from each brood, (v) number of fathers and the reproductive skew among them, and (vi) prior probability of multiple paternity based on other biological data or previous independent genetic analyses. The mean f_{mm} and 95% confidence intervals were calculated for each population using allele frequencies for

Ham 6 and *Ham 44*, 36 broods, 30 eggs per brood and seven different combinations of number of fathers and relative contributions: two fathers contributing 50:50, 90:10 or 95:5; three fathers contributing 33.3:33.3:33.3, 60:30:10, 50:40:10 or 50:45:5. Because the prior probability distribution of multiple paternity was unknown, we assumed it followed a uniform distribution (Neff *et al.* 2001, 2002). Note that we refer to f_{mm} as the frequency of multiple paternity, whereas Neff & Pitcher (2002) originally defined it as the frequency of multiple mating, where the term 'mating' was used to reflect fertilization and not only copulation. We feel this distinction is important because the presence of a female sperm-storage organ and the incubation of fertilized eggs on the female potentially allow to assess both the number of female mates and the identity of the fathers for wild-mated females in lobsters as in brachyuran crabs (Urbani *et al.* 1998; Roy 2003), which are both critical informations for understanding mating systems (Shuster & Wade 2003).

The nonparametric two-tailed Wilcoxon rank sum test, performed with the software package JMP 5.1 (SAS Institute 2003), was used to contrast the CL of females with single and multiply sired clutches. A Monte Carlo simulation (see Roff & Bentzen 1989), using the REAP 4.0 MONTE program (McElroy *et al.* 1992), was performed to test for heterogeneity among population frequency of multiply sired clutches. A G-test for goodness of fit was used to test the null hypothesis that fathers contributed equally to multiply sired broods.

Results

The set of microsatellites used in this study provided considerable power for detecting multiple paternity in broods in each of the studied populations (Table 3). Obviously, the *PrDM* improved as the sample size increased. However, a sample of only 20 eggs was sufficient to obtain a *PrDM* of 86% with the four loci under the assumption of skewed paternity, and of 99.8–99.9% under the assumption of

Table 3 Probability of detecting multiple paternity (*PrDM*) for the four microsatellite loci assuming multiply mated broods to be the product of either two fathers with equal paternity (50:50; first number) or two fathers with skewed paternity (90:10; second number) and various sizes of egg samples (*n*). MI, Magdalen Islands; AI, Anticosti Island; GMI, Grand Manan Island. See text for model and simulation details

Eggs <i>n</i>	Site		
	MI	AI	GMI
100	0.999/0.999	0.999/0.999	0.999/0.998
50	0.999/0.989	0.999/0.989	0.999/0.990
30	0.999/0.944	0.999/0.942	0.999/0.944
20	0.998/0.858	0.999/0.860	0.999/0.856

Table 4 Information on female American lobster expressing multiple paternity at Magdalen (MI) and Grand Manan (GMI) islands. Female carapace length (CL, in mm), number of paternal alleles for each locus and the minimum number of fathers are shown. The percentage of progeny for the primary, secondary and tertiary father (separated by colons) were obtained with loci *Ham 6* and *Ham 44* (see text for details)

Female identity	Female CL	Number of paternal alleles				Inferred no. of fathers	Percentage progeny for fathers
		<i>Ham 6</i>	<i>Ham 10</i>	<i>Ham 21</i>	<i>Ham 44</i>		
MI08	75.9	4	2	2	4	2	70:30*
MI30	75.6	3	2	3	4	2	60:40
MI22	77.6	4	3	4	2	2	77:23*
MI88	86.1	4	2	2	4	2	53:47
GMI01	96.8	4	2	3	3	2	83:17*
GMI02	111.3	4	2	4	4	2	90:10*
GMI03	128.6	2	3	4	3	2	53:47
GMI08	133.3	3	2	2	4	2	87:13*
GMI14	114.9	3	2	3	4	2	90:10*
GMI26	119.6	4	4	2	2	2	60:40
GMI31	137.4	4	3	3	4	2	63:37
GMI35	138.1	4	2	3	3	2	57:43
GMI16	134.4	5	4	4	6	3	73:17:10*
GMI36	94.6	5	4	2	5	3	57:30:13*

*Skewed paternity, G-test of goodness of fit, $P < 0.05$, $n = 30$.

equal male contributions to progeny. With a sample size of 100 eggs, such as we used in our study, the *PrDM* values were 99.8–99.9% for the three populations regardless of the assumption. Consequently, genotype profiles obtained for pooled egg samples and individual eggs allow us to make reliable inferences on the frequency and intensity of multiple paternity for lobster.

Overall, multiple paternity (i.e. > 2 paternal alleles at ≥ 2 loci) was detected in 13% of the 108 examined broods. For each locus, genotype profiles of the eggs from the 1080 sampled pleopods all contained maternal alleles along with 1–6 paternal alleles. Mutation or null alleles are unlikely to have biased our estimates of multiple paternity, because maternal alleles were never lacking in the broods. In each case of multiple paternity, the genetically different offspring were detected on each of the female’s 10 pleopods. The analysis of the 30 individually genotyped eggs from each of the multiply sired broods ($n = 14$) revealed the same maternal and paternal *Ham 6* and *Ham 44* alleles that were detected in the sample of pooled eggs, but allowed us to evaluate the relative offspring contributions from the various fathers (Table 4). Among cases of double paternity, one father contributed > 80% of the assayed progeny in four broods (GMI01, GMI02, GMI08 and GMI14), one father contributed 60–80% of the progeny in five broods (MI08, MI30, MI22, GMI26, GMI31), and two fathers contributed almost equally in three broods (MI88, GMI03, GMI35). In the two cases of triple paternity (GMI16, GMI36), there was a predominant father that contributed 57–73% of progeny while the tertiary father contributed only 10–13% of the

progeny. The relative contribution of fathers to the broods departed significantly from equality in half of the cases of double paternity and in both cases of triple paternity (Table 4).

Differences in the frequency and intensity of multiple paternity were apparent among sampling sites (Table 4). No case of multiple paternity was detected at Anticosti Island, four cases (11.1%) of double paternity were detected at the Magdalen Islands, and eight cases (22.2%) of double paternity and two cases (5.6%) of triple paternity were detected at Grand Manan Island. The null hypothesis that frequency of multiple paternity is independent of sampling site was rejected ($\chi^2 = 3.19$, $P = 0.036 \pm 0.002$; 10 000 randomizations). The smallest mean values of model-based estimates of f_{mm} (Table 5) were 11% (95% CI: 2–24%) at the Magdalen Islands, 2% (0–9%) at Anticosti Island, and 26% (13–42%) at Grand Manan Island, assuming ≤ 3 fathers contributed equally to the broods. At the other extreme of the simulation range, we obtained maximum mean values that were, respectively, 17% (5–32%), 2% (0–12%) and 32% (15–52%) assuming that two fathers had a reproductive skew of 95% and 5%.

Females with a multiply sired clutch tended to be smaller (MI: 78.8 ± 2.5 mm CL; GMI: 120.9 ± 16.2 mm CL) than those with a singly sired clutch (MI: 94.2 ± 4.0 mm CL; GMI: 133.1 ± 23.7 mm CL). However, the size difference between the two groups of females was marginally not significant at the Magdalen Islands (Wilcoxon rank sum test, $z = -1.84$, $P = 0.066$) and not significant at Grand Manan Island GMI: $z = -1.04$, $P = 0.297$).

Table 5 Summary of the estimated frequency of multiple paternity with 95% confidence intervals (in brackets) for American lobster from Magdalen (MI), Anticosti (AI) and Grand Manan (GMI) islands. Seven scenarios were considered for the number of fathers and their reproductive skew (in parentheses). See text for model and simulation details

Sites	Number of fathers (skew)						
	2 (50:50)	2 (90:10)	2 (95:5)	3 (33.3:33.3:33.3)	3 (60:30:10)	3 (50:40:10)	3 (50:45:5)
MI	13 [4–25]	14 [4–26]	17 [5–32]	11 [2–24]	13 [3–25]	12 [3–24]	13 [3–25]
AI	2 [0–9]	2 [0–10]	2 [0–12]	2 [0–9]	3 [0–14]	2 [0–9]	2 [0–9]
GMI	25 [12–40]	26 [13–43]	32 [15–52]	26 [13–42]	29 [15–44]	28 [15–43]	29 [15–45]

Discussion

While it has long been generally recognized that male lobster can be quite polygynous (Herrick 1911; Templeman 1934; Cowan & Atema 1990; Waddy & Aiken 1991), the range of female mating behaviours has not been fully resolved. Laboratory evidence of promiscuity, most of it anecdotal, exists for females (e.g. Easton 1976; Waddy & Aiken 1990, 1991; Snyder *et al.* 1992) and multiple paternity was inferred from allozyme electrophoresis of one female (Nelson & Hedgecock 1977). The present study, as Jones *et al.*'s (2003) report of one case of double paternity using microsatellite loci, unambiguously shows that female lobster may, between molting and one or more successive ovipositions, mate with more than one male.

Very little information exists on the frequency of multiple paternity in wild crustacean populations, but it appears to vary among species. The overall 13% occurrence for lobster based on allele counts in the present study is small when compared to the crayfish, *Orconectes placidus* (60% of 15 broods, Walker *et al.* 2002), the Norway lobster, *Nephrops norvegicus* (54.6% of 11 broods, Streiff *et al.* 2004) and the porcelain crab, *Petrolisthes cinctipes* (80% of 10 broods, Toonen 2004), but is high when compared to primiparous females of the snow crab *Chionoecetes opilio* (0% of seven broods, Urbani *et al.* 1998; 0% of five broods, Sainte-Marie *et al.* 1999; 3.8% of 79 broods, Roy 2003). Paternity analyses will underestimate the frequency of female promiscuity if sperm from one of several mates is exclusively favoured, for example by its localization in or on the female or by hypothetical female postcopulatory (cryptic) choice (for crustacean examples, see Sévigny & Sainte-Marie 1996; Thiel & Hinojosa 2003). Of the total 91 female snow crabs examined in the three studies mentioned, 88% had mated with two to six males based on the analysis of spermathecal content but the proportion of broods expressing multiple paternity was very small because sperm from one male had precedence over other sperm because of strategic placement within the spermatheca or because additional sperm were acquired after eggs were fertilized (Urbani *et al.* 1998).

The relatively high incidence of single paternity in lobster can suggest that females are generally monandrous between molting and the following intermolt oviposition(s). For several reasons, this hypothesis seems more likely than the alternative hypothesis that females are typically polyandrous but express single paternity because of effective sperm precedence mechanisms or female postcopulatory choice. First, the lobster seminal receptacle is not nearly as complex and expandable as the spermathecae of brachyuran crabs that exhibit a high frequency and degree of female promiscuity (see Diesel 1991; Sainte-Marie *et al.* 2000), so discrete or orderly storage of multiple ejaculates that could favour sperm precedence or cryptic choice in polyandrous females seem improbable. Second, in the lobster, recently introduced ejaculate when provided in sufficient quantity often forms a protruding sperm plug that may prevent for some time any further mating by the female (Templeman 1934, 1936). Third, given that sex roles are partially reversed in the lobster and that it is the female that actively seeks out and attempts to mate with a preferred male (Atema *et al.* 1979; Cowan & Atema 1990), there would seem to be no need for the females to resort to covert strategies for selecting better mates (or genes) which is an often cited explanation for female promiscuity (e.g. Trivers 1972; Zeh & Zeh 1996, 2001). We therefore propose, as Waddy & Aiken (1990) before, that female promiscuity between molting and oviposition(s) is limited to cases when sperm reserves are too small to ensure full fertilization of the upcoming clutch (see succeeding discussion), i.e. it protects against sperm limitation (e.g. Anderson 1974; Walker 1980; Pitnick 1993).

Additionally, the fertilization process in the lobster does not seem to be suitable for sperm precedence or selection. The sperm are not motile, as in all other decapod crustaceans (Krol *et al.* 1992), and fertilization occurs externally as described by Aiken *et al.* (2004). Female muscular contractions are involved in the release of sperm from the central seminal receptacle and, in our opinion, they could possibly cause sperm to mingle when more than one ejaculate is stored. The ova are extruded through paired oviducts and channeled over the posterolateral grooves of the central

seminal receptacle. The intermixing of gametes and the subsequent displacement of fertilized ova for attachment to pleopods are ensured by movements of the first pair of pleopods in an alternating process that lasts up to several hours. Our observation that the differently sired progeny were distributed across all pleopods can suggest that sperm from different fathers mingled before or during the fertilization process. Interestingly, females of the Norway lobster *N. norvegicus* that expressed multiple paternity had broods that were sired usually by two males, and in one case by three males, but relative contribution of competing fathers to progeny never differed significantly from equality (*G*-test performed by us on data in Table 3 of Streiff *et al.* 2004). In the multiple paternity cases of our study, slightly more than half of the broods exhibited skewed paternal contributions while the others did not. Such a pattern could be the result of a raffle competition (Dickinson 1986; Parker 1990) whereby paternity is allocated in proportion to the size (number of sperm) of the various ejaculates (e.g. Bissoondath & Wiklund 1997; Sauer *et al.* 1999). Differences in the size of competing ejaculates held by female lobster may reflect mate size or quality (MacDiarmid & Butler 1999; Gosselin *et al.* 2003) or the partially depleted state of older relative to newer ejaculates in sequentially spawning females.

The lack of multiple paternity in the Anticosti population, and its occurrence in the Magdalen Islands population and in the migrating component of the Grand Manan population might be related to differential exploitation rates and sperm limitation. The ratio of large males to receptive females may be smaller in heavily exploited populations (Magdalens, maybe Grand Manan) than in lightly exploited populations (Anticosti), forcing females, and perhaps especially the smaller less fecund and therefore less attractive females (as hinted by the smaller mean size of females with multiply sired broods within each of the Magdalens and Grand Manan populations), to mate with small males that pass quantities of sperm that are insufficient for fertilizing the female's entire clutch. Under these circumstances, females may quickly attempt to mate with another male (Easton 1976; Gosselin *et al.* 2003) and the sperm plug may be too small to effectively prevent an additional copulation. This interpretation is consistent with observations on the lepidopteran *Pieris napi* showing that females mated by small males received less sperm and remated more quickly than females mated by large males (Bissoondath & Wiklund 1997). For the time being, however, the link between the occurrence of female promiscuity (i.e. multiple paternity) and sperm limitation in the lobster is still tenuous. Unfortunately, the few other studies on multiple paternity in lobster either did not provide a measure of its frequency within populations (total number of females examined for multiple paternity was not specified in Nelson & Hedgecock 1977 or Jones *et al.* 2003) and/or

information on the size of females and number of eggs that were examined (Tam & Kornfield 1996). Further research will be necessary to assess the significance of multiple paternity in lobster and this work should consider the frequency of female promiscuity by genotyping the contents of the seminal receptacle of pre- and postoviposition females and the factors of promiscuity and multiple paternity, such as female size and sperm allocation by first mate, in a comparative multipopulation framework.

To conclude, our genetic parentage analysis has demonstrated that (i) overall, 13% of the broods were multiply sired, in general by two males but occasionally by three males; (ii) multiple paternity was not equally frequent among populations; (iii) the offspring from different fathers were distributed across all the female's pleopods; and (iv) the contributions of different fathers to a brood were significantly skewed in over half of the cases.

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This research comprises part of Thierry Gosselin's MSc. thesis, which was concerned with the mating system and potential for sperm limitation in American lobster. TG is currently a doctoral student at Université du Québec à Rimouski examining the genetic basis for multiple mating by female snow crabs. Bernard Sainte-Marie and Louis Bernatchez were advisers for Thierry Gosselin's MSc thesis. BSM's research focuses on the mating system, reproductive biology, population dynamics, and ecology of marine decapod crustaceans. LB's research interests relate to the understanding of patterns and processes of molecular and organismal evolution, as well as their significance to conservation.
