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The effects of controlled propagation on an endangered species: genetic differentiation and divergence in body size among native and captive populations of the Socorro Isopod (Crustacea: Flabellifera)

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Abstract

The endangered Socorro Isopod, *Thermosphaeroma thermophilum*, is endemic to a single thermal spring in Socorro, NM. This species is cannibalistic, with males more cannibalistic than females, and with females and juveniles more vulnerable than males as prey. In 1990, the New Mexico Department of Game and Fish, created the Socorro Isopod Propagation Facility (SIPF) near the natural habitat, Sedillo Spring (SS), to increase total population size and to examine the effects of habitat heterogeneity on population growth. We report the genetic and morphological effects of this experiment, using the natural population as a control. Captive subpopulations experienced bottlenecks of known intensity and duration, as well as different intensities of cannibalism. Using 57 AFLP markers, we show that in 6 years (1990–1996), captive subpopulations diverged significantly from the natural population. Also during this 6-year period, body lengths of captive isopods diverged nearly 2-fold from the natural population, evidently because cannibalism and thus selection favoring large size was more intense in captive subpopulations than in nature. This hypothesis is supported by the fact that cannibalism and the apparent response to selection on body size became variable among captive subpopulations when physical structure was added to three of the four SIPF pools in April 1997. As expected if cannibalism was the source of selection for large body size, by August 1998 (15 months = 7–8 generations), the rate at which body size increased became inversely proportional to the amount of physical structure within pools. Although we are unable to separate the specific effects of population subdivision and cannibalism, our results show that these conditions in combination caused rapid changes in genetic variation and the external morphology of these captive subpopulations. Our results have important implications for future attempts to manage and propagate endangered species.

Introduction

Predation is widely recognized as a powerful evolutionary force (May 1973, 1981; Pianka 1994). The specific effects of predation may vary depending on the predator, as well as on the life stage of prey that predators prefer. Selection is generally expected to favor rapid growth in vulnerable life stages, or large size overall, because such individuals are too costly to overpower.

Culling of females and juveniles is expected to have a greater negative effect on population size than removal of males or individuals from older age classes. Not only does this practice prevent pre-reproductive individuals from reproducing, but reducing the number females also reduces the effective population size. If the mating system of the prey population involves male mate guarding, the effective population size can be further reduced, particularly if large males have an advantage over smaller males in acquiring and retaining their mates (Shuster and Wade 2003). Such reductions in effective population size are expected to accentuate the effects of genetic drift, and thus enhance the negative as well as the possible adaptive effects of genetic bottlenecks (Whitlock and Barton 1997).

The selective effects of cannibalism appear to be particularly intense. Cannibals often prefer certain conspecific genotypes or life stages (Mayes and Englert 1984; Goff and Stevens 1995; Summers and Amos 1997), and some habitat locations are more vulnerable to invasion by cannibals than others. If habitat preferences as well as tendencies toward cannibalism are heritable, cannibalism can rapidly lead to changes in the preferred habitats of vulnerable life stages (Jormalainen and Shuster 1997), as well as in the population frequencies of cannibals (Shuster and Wade 2003). Like sexual selection, sex specific cannibalism can also cause deviations in population sex ratio that enhance the intensity of selection on particular phenotypes (Elgar et al. 1990). Moreover, if cannibalism removes particular life stages, genotypes or members of one sex, cannibalism is expected to reduce effective, as well as actual population size.

Eight described species of *Thermosphaeroma* (Crustacea: Flabellifera) isopods inhabit isolated thermal springs in New Mexico and Texas in the United States, and in the Mexican states of Chihuahua, Coahuila, Durango and Aquascalientes (Shuster 1981a, b; Bowman 1981, 1985; Davis 1996; Schott 2000). *Thermosphaeroma thermophilum* inhabits a single thermal spring near Socorro, NM (Shuster 1981a). Its limited range and small population size (< 3000 individuals) has led to the species' "endangered" status (Federal Register 1977). Jormalainen and Shuster (1997) examined microhabitat use, cannibalism and individual responses to conspecifics in *T. thermophilum*. In nature, juveniles (mancas) and females were found mainly on vegetation; males were found mainly on sediments. In laboratory containers without refugia, males cannibalized females, both males and females cannibalized mancas, and mancas cannibalized each other, even in the presence of alternative food. In containers provided with refugia, mancas avoided adults. Thus, cannibalism in *T. thermophilum* appears to have generated age-, size-, and sex-specific predation risks that are responsible for microhabitat segregation between mancas and adults, and between males and females. Details of this species life history and breeding biology are available in Shuster (1981a, b), Jormalainen and Shuster (1999) and Jormalainen et al. (1999).

In 1990, the New Mexico Department of Game and Fish constructed eight concrete pools near Sedillo Spring (SS) in Socorro, NM, to increase available habitat for *T. thermophilum* (USFWS 1982; Federal Register 2000). Pools were designed to replicate the dimensions of the natural habitat and were supplied with water from the natural spring. Each series of pools (North 1–4; South 1–4) was connected by 3.2 cm PVC pipe. Approximately 300 isopods collected from SS were introduced to each of the two series of the SIPF in September 1990. Approximately 500 isopods of this total sample were maintained at the University of New Mexico before introduction (Lang, in preparation). By spring 1995, the four isopod subpopulations inhabiting the North series had stabilized whereas the South series had gone extinct. Standardized monthly population censuses began in July 1995, with collection of monthly voucher material commencing in May 1996. Between 1990 and 1996, Socorro Isopod Propagation Facility (SIPF) isopods were isolated from the natural population, but their body sizes were not monitored.

Between September 1990 and April 1997, SIPF pools contained no physical structure other than that provided by algae growing on pool walls and a layer of fine substrate in each pool, conditions similar to those of SS, although this spring is shallower by 10–20 cm, contains rocks and pebbles and supports emergent vegetation within the main pool and in a shallow efferent stream $\sim$15 m in length (Shuster 1981b). As part of an experiment to identify the effects of habitat structure on isopod numbers in artificial habitats, and to test the predictions of Jormalainen and Shuster (1997), three treatments were introduced in SIPF north pools in April 1997 (Lang 1998). These modifica-

tions consisted of plants added to N2, rocks added to N3 and both plants and rocks added to N4. No modifications were made to N1.

Here, we report the evolutionary effects on an endangered species, of four replicated population bottlenecks of known intensity and duration, combined with differential cannibalism on females and juveniles within these subpopulations. We show that in less than a decade of species management, captive subpopulations of the endangered freshwater isopod, *T. thermophilum*, have undergone significant genetic divergence from the natural population of this species. Based on the analysis of 57 AFLP markers, captive subpopulations of the Socorro Isopod diverged genetically from the natural population in less than 6 years. This genetic divergence was primarily influenced by 2 of the 57 markers examined, indicating that natural selection may have influenced patterns of AFLP diversity between the natural and captive populations. Over this same 6-year period, body lengths of captive isopods diverged significantly from those in the natural population, with average male and female body size increasing as much as twofold compared to the natural population. This result is consistent with the hypothesis that natural selection associated with cannibalistic behavior ultimately favored large body sizes within captive populations.

Methods

Collection of specimens

Voucher specimens were collected from each of the four pools that make up the North series of the SIPF as well as from SS each month beginning in May 1996 (Table 1) using a $5 \times 10 \times 3$ cm sampler,

Table 1. Collections of *T. thermophilum* from Sedillo Spring, Socorro, NM, May 1996 to July 1998; see text for explanation of how samples were pooled for statistical analysis

Date	North pool 1			North pool 2			North pool 3			North pool 4			Sedillo Spring		
	Males	Females	Undiff.	Males	Females	Undiff.	Males	Females	Undiff.	Males	Females	Undiff.	Males	Females	Undiff.
May-96	2	4	3	3	6	1	0	9	2	0	5	6	3	6	0
June	1	3	5	1	5	3	0	8	1	0	7	2	1	7	2
July	3	7	0	2	5	3	4	2	4	0	7	1	5	5	0
August	0	9	1	1	7	2	0	9	1	1	8	1	4	3	3
September	1	4	4	1	6	4	2	7	2	2	4	4	3	7	0
October	2	8	0	2	8	1	0	4	5	4	4	1	6	3	2
November	0	9	3	0	10	0	0	10	0	0	10	0	2	6	2
December	3	3	4	2	8	0	0	9	0	3	6	0	1	5	3
January-97	1	5	5	2	7	3	1	9	1	1	8	1	1	5	4
February	2	14	4	2	16	4	2	8	12	4	12	4	10	0	8
March	3	5	0	2	9	1	1	3	6	0	6	1	6	2	2
April	0	5	4	0	6	3	0	8	2	0	6	7	1	4	4
May	1	5	4	0	9	1	1	5	4	2	7	2	2	6	2
June	1	5	3	0	7	4	0	2	8	5	3	1	2	3	6
July	0	5	5	4	1	4	3	7	0	1	6	4	3	2	3
August	2	8	0	1	8	1	1	6	2	1	9	0	2	7	1
September	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
October	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
November	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
December	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
January-98	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
February	1	7	1	1	8	2	1	6	2	3	7	0	2	2	4
March	3	6	1	2	8	0	2	8	0	2	7	0	5	3	2
April	2	7	1	5	5	0	2	8	0	3	7	0	2	8	2
May	2	7	0	4	5	0	2	7	0	3	6	0	3	4	2
June	5	4	1	6	4	0	3	4	2	6	1	1	6	1	3
July	5	5	0	4	6	0	6	3	1	5	5	0	6	3	1
Totals	40	135	49	45	154	37	31	142	55	46	141	36	76	92	56

and were identified by sex and reproductive condition (Shuster 1981a; Jormalainen and Shuster 1997). Each voucher contained ~10 individuals, including the largest male found in five collections with the sampler, as well as several smaller individuals, usually females, but occasionally including undifferentiated mancas. All vouchers were preserved in 70% ethanol and stored in the Invertebrate Museum at Northern Arizona University. We collected 22 specimens per month from each of the North series of SIPF pools and from the natural habitat between May 1996 and July 1998 (Table 1). Specimens collected for DNA analysis in May 1996 consisted of 50 isopods from each tank in the North series and from SS. All collections complied with state and federal regulations and were preserved in 95% ethanol.

Genetic analyses

DNA was isolated from individual isopods using a phenol/chloroform organic extraction protocol (Sambrook et al. 1989) and purified with Qiagen DNeasy Tissue Kits. Amplified Fragment Length Polymorphisms (AFLP) for each individual was resolved as in Vos et al. (1995) and Miller et al. (2001). We used eight primer pairs for the second selective PCR amplification with selective nucleotides: [*Eco* + AGG/*Mse*+] ACG, ATG, AAG, ATC, AAC, and [*Eco*+ACG/*Mse*+] ATG, AAG, ATC. Gel analysis followed the second selective PCR according Vos et al. (1995) and Travis et al. (1996). Multilocus marker phenotypes were recorded based on the presence or absence of unambiguous, easily scored markers for 137 individuals: 22 from N1, 27 from N2, 28 from N3, 30 from N4, and 30 from SS.

Within-location genetic variation was characterized using Nei's (1978) unbiased expected average heterozygosity (H) and percent polymorphic loci (P). Loci were considered polymorphic if the frequency of the most common allele was ≤ 0.95. Using TFPGA (Miller 1997a) and the allele frequency estimator of Lynch and Milligan (1994), we obtained allele frequency estimates from each dominant AFLP marker, assuming each dominant marker to be a 2-allele Mendelian locus in H–W proportions. Estimates for H ($\pm$95%CI) were based on 1000 bootstrap replicates (Efron 1979; Efron and Tibshirani 1993; MPM). We also tested for differences in marker phenotype variation among collection locations using Homogeneity of Molecular Variance (HOMOVA; Stewart and Excoffier 1996; and WINAMOVA 1.55, Excoffier 1992), which calculated a Bartlett's heteroscedasticity statistic (B) using 1000 permutations. Data files were produced using AMOVA-PREP (Miller 1997b).

Because our sampling of collection locations was complete (i.e., these organisms only exist in the natural habitat and four experimental tanks), we considered the bias-corrected, random model statistical procedures such as Weir and Cockerham's (1984) θ and the AMOVA procedure of Excoffier et al. (1992) for dominant marker DNA fingerprints (Huff et al. 1993) inappropriate. Since all *T. thermophilum* locations were included in analyses, we adopted two fixed model procedures to test for differences in marker phenotype frequencies of sampling locations. First, we used TFPGA to perform a variation of Fisher's exact test (Fisher 1954; Raymond and Rousset 1995), which tested for locus-specific differences in marker phenotype frequencies from each locus as 5×2 contingency tables (five locations, two marker states per locus). We compared all pair-wise combinations of populations at each locus using Fisher's combined probability test (Fisher 1954; Sokal and Rohlf 1995) to generate global estimates of the significance over loci. P-Value estimates for each contingency table were obtained by using 10 batches of 2000 permutations per batch (20,000 total permutations) and an initial 1000-step dememorization procedure. The use of batching permitted calculation of standard errors of p-value estimates.

Second, we used a variation of the Mantel matrix correlation analysis (Mantel 1967; Sokal and Rohlf 1995) in MANTEL-STRUCT (Miller 1999) to detect within and among-location genetic differences in individual AFLP profiles. We used the Dice coefficient (Dice 1945; Miller 1999) to create the off-diagonal half matrix of inter-individual similarities. The standard product-moment correlation of the similarity matrix and a binary matrix containing unity (1) in congruent within location positions and 0 in congruent between location positions was calculated to serve as an indicator of genetic differentiation. The significance of the overall correlation was assessed by randomly permuting rows and columns of the binary matrix 1000 times (Mantel 1967). Using MAN-

TEL-STRUCT, we tested for overall differences among the five sampling locations as well as for differences in each pair-wise combination of sampling locations. Because our analyses suggested that the natural and captive populations displayed relatively high levels of divergence (see section ''Results and Discussion''), we also performed the two analyses described above to compare the natural population to the pooled samples from all four of the captive habitats. In the MANTEL-STRUCT procedure, analyses were conduced for each locus individually to assist with the identification of specific markers that contributed to the pattern of divergence.

Finally, because the linear nature of the connections between the four captive subpopulations suggests possible isolation by distance, we tested for such a pattern using a Mantel test in TFPGA, in which pair-wise genetic distances of sampling locations were calculated using the coancestry distance of Reynolds et al. (1983). This measure is appropriate under a pure genetic drift scenario (Reynolds 1983; Weir 1996), a case that is likely given the short period of time that these locations have been isolated (6 years). We plotted pair-wise genetic distances of sampling locations against pair-wise physical distances between sampling locations. Physical distances were defined in units of ''steps,'' or number of connections separating each pool. We calculated the correlation of the genetic and physical distances of these two matrices and evaluated its significance using 1000 randomization events, and we used a UPGMA analysis of pair-wise coancestry distances of all five sampling locations to obtain a graphical representation of collection location genetic dissimilarities, using a bootstrap sampling procedure over loci (sensu Felsenstein 1985) to record the proportion of bootstrap replicates that recovered clusters similar to those observed based on the original data.

Body length analysis

We posed four questions related to changes in body size in natural and captive isopod populations. Our first question was, what changes in body size occurred between 1990 and 1996? We addressed this question in three ways: (a) To determine whether differences in body length among individuals from natural and captive subpopulations were attributable to collection date, pool of origin and ''sex'' (male, female, and manca), and to justify post-hoc comparisons to identify possible causes for inter-population differences in body length that occurred between 1990 and 1996 also 1996 and1998, we used a linear model to identify the main effects and first-order interactions of these three factors on the entire sample of isopods collected during 1996–1998. (b) To provide a standard against which possible changes in body length among captive populations could be compared, we compared the body lengths of males and females collected in 1978 (Shuster 1981a), in 1995 (Jormalainen and Shuster 1997), and in our pooled collections from 1996 and 1998. (c) To identify specific changes in the body lengths of males, females, and mancas that occurred within captive subpopulations between 1990 and 1996, we used a 2-level linear model to examine the effects of pool of origin and sex on the body lengths of all isopods collected between May 1996 and April 1997. Because this result was significant (see section ''Results''), we used three separate 1-level linear analyses to determine within each sex, whether and to what degree the body lengths of individuals in captive subpopulations had diverged from the natural population. We adjusted the level of significance for our analyses using a Bonferroni correction ($\alpha = 0.05/(2+3) = 0.01$).

Our second question related to changes in body size in natural and captive isopod populations was, did the body sizes of males, females, and mancas change in the same way between 1996 and 1998? We subdivided our 1996–1998 dataset by sex (males, females, and mancas) and examined the effect of pool of origin and collection date on each life stage using a 2-level linear model without interaction (because these subsets were small). We adjusted our analyses using a Bonferroni correction ($\alpha = 0.05/3 = 0.017$). To visualize the pattern of this variation within and among life stages as it occurred across all collections and habitats, and in particular to examine of the degree to which captive populations had diverged from the natural population, we plotted average the body lengths ($\pm$95%CI) of males, females and mancas by habitat and collection date, before and after physical structure was added to pools N2–N4 (Figure 4).

Our third question was, can differences in body size occurring between 1996 and 1998 be attributed

to changes in the physical structure of the habitats containing captive populations? To address this question, we calculated the linear regression of body length on sampling date for males, females and mancas in N1–N4 and in SS. Because our 15 analyses represented subdivision of the total sample by sex ($N = 3$) as well as by collection site ($N = 5$), we adjusted the level of significance for our analyses using a Bonferroni correction ($\alpha = 0.05/15 = 0.003$).

Our fourth question was, can evidence of significant selection on body length be detected within habitats containing captive subpopulation between 1996 and 1998? We predicted that the intensity of selection on body size within the captive populations after April 1997 to follow the order N1 > N3 > N2 > N4. To test this prediction, we identified each of our 11, 2-month pooled samples as ''generations,'' to simulate the estimated 2-month generation time of this species (Shuster 1981a). We next calculated linear regressions of average body lengths for males, females, and mancas collected from each captive population, on the average body lengths of males, females, and mancas collected from SS at the same time, respectively. We calculated the residuals for each of these three regressions, and then calculated the linear regression of each set of residuals on the pooled-sample collection dates. This procedure provided us with a powerful statistical test for detecting a change in phenotype resulting from selection, in the presence of between-generation environmental variation (Muir 1986; Wade et al. 1996). Here, we compared deviations in body sizes of males, females, and mancas in each captive population with those in a ''control'' population (SS). This approach allowed us to detect changes in body length in each population that occurred over time, and account for the effects of environmental variation within the captive and natural populations.

Results

Genetic differentiation among populations

In total, the AFLP procedure provided 57 markers that varied among the five collection sites. We detected no significant differences in average within-location genetic variation among these markers, Estimates of H using AFLPs exceeded 0.25 for all habitats (mean ± SD = 0.28 ± 0.02), with overlapping 95% CI. Percent polymorphic loci estimates ranged from ~81% to 91%. Heteroscedasticity of raw marker phenotype data was non-significant (Bartlett's test; $B = 0.14$, $p = 0.053$), providing further evidence for a lack of detectable differences in within-location genetic variation.

Tests for genetic differentiation of sampling locations indicated that the four captive subpopulations were genetically differentiated from the natural habitat. In single-locus 5×2 contingency tables, 13 of the 57 markers differed significantly in frequency among collection locations at the $\alpha = 0.05$ level and Fisher's combined probability test over loci was highly significant ($\chi^2 = 245.84$, df = 114, $P < 0.0001$). Pair-wise analysis of all sampling locations suggested that the main source genetic differences existed between the natural population and all of the captive subpopulations (Table 2). Significant differences were observed between the natural habitat and each of the four captive habitats after a sequential Bonferroni procedure (Sokal and Rohlf 1995), but no differences were detected among the four captive habitats, a result confirmed by matrix correlation. In the simultaneous analysis of all five-collection locations, the correlation of the inter-observational similarity matrix and congruent binary matrix was highly significant ($r = 0.06$, $P < 0.001$). The analysis of all pair-wise combinations of collection locations revealed patterns similar to those seen when data were analyzed using contingency tables (Table 3). Highest correlations, all > 0.2 and all highly significant ($P < 0.001$), were observed between the natural population and each of the captive subpopulations. Lower, generally non-significant correlations < 0.035, were observed for all contrasts of the captive habitats save for the comparison of N2 and N4 ($r = 0.06$, $P = 0.003$).

In pooled samples from all four captive subpopulations, when compared to the natural subpopulation, two AFLP markers contributed disproportionately to the differentiation of the natural and captive subpopulations (Table 4, Figure 1). Markers ACG-ATC:168 and AGG-ACG:276, while polymorphic within the natural subpopulation, were absent from individuals in the captive subpopulations with the exception of a single female that displayed both marker phenotypes (Table 4). The single-locus correlation coefficients obtained from MANTEL-STRUCT

Table 2. Summaries of the results of per-locus contingency table analyses conducted on all pair-wise combinations of collection locations

Sampling location	North pool				Sedillo Spring
	1	2	3	4	
NP 1	–	1	0	0	8
NP 2	P = 0.9966 χ^2 = 77.25 df = 114	–	5	4	7
NP 3	P = 0.7096 χ^2 = 105.23 df = 114	P = 0.6052 χ^2 = 109.37 df = 114	–	6	7
NP 4	P = 0.9574 χ^2 = 89.36 df = 114	P = 0.1937 χ^2 = 126.83 df = 114	P = 0.9149 χ^2 = 93.93 df = 114	–	7
Sedillo Spring	P < 0.0001 χ^2 = 178.50 df = 114	P < 0.0001 χ^2 = 195.21 df = 114	P < 0.0001 χ^2 = 176.89 df = 114	P = 0.0148 χ^2 = 149.32 df = 114	–

Values below the diagonal are the results of Fisher's combined probability test applied to *P*-values from the analysis of all 57 loci. Values above the diagonal are the number of loci out of 57 that significantly differed between collection locations at 0.05 level (listed here only to serve as an indicator of genetic differentiation).

Table 3. Values in bold type along the diagonal of the matrix indicate average between-location genetic similarities of individuals while values below the diagonal indicate average genetic similarities of individuals from each pair-wise combination of sampling locations

Sampling location	North pool				Sedillo Spring
	1	2	3	4	
NP 1	**0.6254**	r = −0.0097 P = 0.5874	r = 0.0216 P = 0.2298	r = 0.0238 P = 0.1958	r = 0.2529 P < 0.001
NP 2	0.6243	**0.6213**	r = 0.0306 P = 0.0609	r = 0.0639 P = 0.003	r = 0.2758 P < 0.001
NP3	0.6181	0.6154	**0.6188**	r = 0.0281 P = 0.0839	r = 0.2312 P < 0.001
NP4	0.6275	0.6188	0.6227	**0.6340**	r = 0.2506 P < 0.001
Sedillo Spring	0.5895	0.5842	0.5879	0.5948	**0.6292**

Values above the diagonal show results of a matrix correlation analysis to determine if there are significant differences in within and between sampling location genetic similarities. The correlation coefficient, *r*, is an indicator of differentiation; its associated *P*-value was obtained from a randomization procedure based on 1000 permutations. See text for further details.

further illustrated that these two markers display a much greater level of divergence than any of the other 55 markers analyzed (Figure 1).

While only four captive subpopulations were examined, our genetic data indicated a general pattern of isolation by distance within this system. The correlation of genetic and physical distances of these habitats, while not significant, was positive and relatively high ($r = 0.67$, $P = 0.16$) and indicated that genetic exchange may occur between adjacent pools. Our use of UPGMA clustering further illustrated genetic patterns within this system, in particular, that the four captive subpopulations were genetically distinct from the natural population (Figure 2). The cluster formed by these habitats appeared in over 98% of the bootstrap samples.

Body length analysis

Our 3-level analysis of male, female, and manca body sizes collected from SS and from North pools

Table 4. AFLP marker phenotype frequencies for markers ACG-ATC:168 and AGG-ACG:275 between the natural and artificial habitats

	AFLP Marker					
	ACG-ATC: 168[a]			AGG-ACG: 276[a]		
	Present	Absent	Total	Present	Absent	Total
Artifical habitat	1[b]	106	107	1[b]	106	107
Natural habitat	22	8	30	25	5	30
Total	23	114	137	26	111	137

[a]Marker names refer to the selective nucleotides used in conjunction with *Eco*RI and *Mse*I primers, respectively, and the markers' sizes in nucleotide bases. [b]The same female from the artificial habitat showed the marker phenotype at both loci.

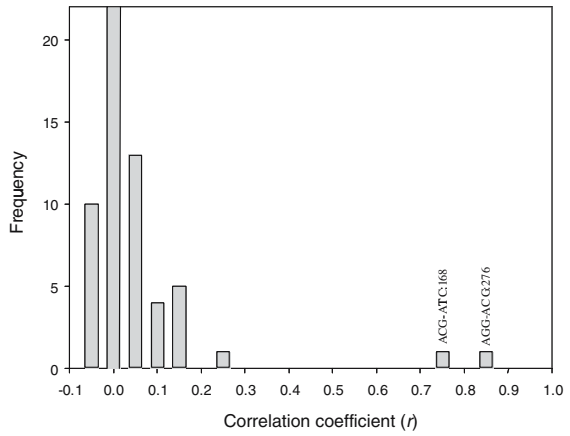

Figure 1. Distribution of per-locus correlation coefficients (r) for the 57 AFLP markers scored in comparisons of all pooled artificial habitat samples and samples from the natural habitat (see text for details). Markers ACG-ATC:168 and AGG-ACG:276 demonstrate the highest levels of genetic differentiation between the natural and captive populations.

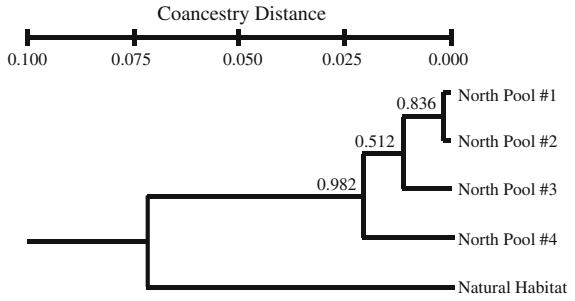

Figure 2. UPGMA dendrogram showing the genetic dissimilarity of each of the five isopod sampling locations. Proportions associated with each node in the tree are the proportion of 1000 bootstrap replicates that produced clusters similar to the one shown.

1–4 between 1996 and 1998 showed significant differences attributable to collection date, pool of origin and sex ($F_{[84,1131]} = 48.4$, $P < 0.001$, $r^2 = 0.812$). All main effects and first-order interactions were significant in this analysis, with the largest components of variance explained by sex ($F_{[2,1131]} = 1135.4$, $P < 0.0001$), pool of origin ($F_{[4,1131]} = 65.5$, $P < 0.0001$) and the interaction between sex and pool ($F_{[8,1131]} = 31.92$, $P < 0.0001$). These results indicated that males, females, and mancas differed from each other in body length, and that the body lengths of these life stages were differentially influenced by the pools from which they were collected. Thus, post-hoc analyses to identify the nature and possible causes for changes in body length among natural and captive subpopulations were justified.

We found no evidence of significant changes in the body lengths of isopods inhabiting SS within the last 20 years [males: *year* (avg. ± 95%CImm, N; Figure 3): 1978 (7.33 ± 1.02 mm, 13); 1995 (6.10 ± 0.14 mm, 229), 1996 (6.38 ± 0.31 mm, 43), 1998 (6.44 ± 0.45 mm, 33); $F_{[3,314]} = 0.05$, $P > 0.75$; females: *year* (avg. ± 95%CImm, N): 1978 (4.73 ± 0.19 mm, 15); 1995 (4.60 ± 0.08 mm, 91), 1996 (4.63 ± 0.11 mm, 53), 1998 (4.40 ± 0.11 mm, 39); $F_{[3,194]} = 0.06$, $P > 0.75$]. Males were on average 1.43 ± 0.10 times larger than females, and the ratio of male to female body length remained essentially unchanged over time. The stability of body size within the natural *T. thermophilum* population justified using animals from this population to identify the degree to which captive isopod populations increased in body size between 1990 and 1998.

Our 2-level analysis to identify changes in body lengths of males, females, and mancas within captive populations between May 1996 and April 1997 was significant ($F_{[3,392]} = 203.9$, $P < 0.0001$),

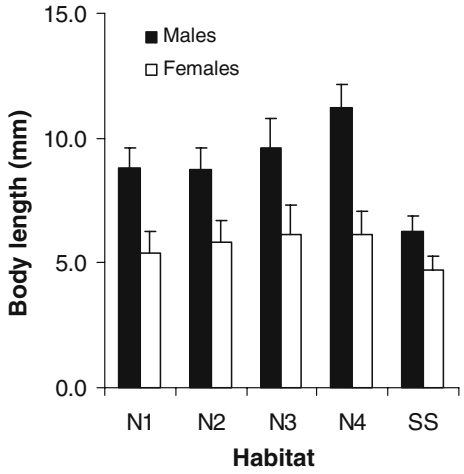

Figure 3. Average (± 95%CI) body lengths of male and female *T. thermophilum* collected between May 1996 and April 1997 from the natural subpopulation (SS) and from captive subpopulations, N1, N2, N3, and N4; males and females in all captive subpopulations increased in body length compared to males and females from the natural subpopulation.

with significant main and interaction effects for location and sex ($F_{[1,\ 392]} = 23.3$, $P < 0.0001$, $F_{[1,392]} = 205.0$, $P < 0.0001$, and $F_{[1,392]} = 15.0$, $P < 0.0001$). This result indicated that body lengths of males, females, and mancas differed within and among pools over this interval, and justified subdivision of our data into three subsets to compare the effect of captive habitats on the body lengths of males, females, and mancas that had occurred between 1990 and 1996.

In separate 1-level analyses, we found significant effects of habitat on body length for males ($F_{[4,63]} = 23.0$, $P < 0.0001$) and females ($F_{[4,252]} = 21.2$, $P < 0.0001$). In both sexes, the effect of habitat was shown in increases in body lengths of individuals in captive populations relative to the body lengths of individuals in SS (Figure 3). Among captive populations, divergence in body length from isopods in the natural population for both males and females appears to have been smallest in N1 and greatest in N4 (Figure 3). These results suggest that conditions favoring large body size affected both sexes within captive subpopulations, and did so in consistent ways within populations. However, among captive subpopulations, large body size appears to have been favored differentially in subtle but significant ways. Thus, while no collections were made between 1990 and 1996, during this interval, conditions within the four captive habitats clearly and consistently favored large body size in both sexes. We found no differences in body length among mancas in captive and natural subpopulations ($F_{[4,72]} = 1.6$, $P = 0.19$), suggesting that the results of selection favoring increased body size in captive subpopulations were primarily realized by adult isopods.

Table 5. Results of 2-way ANOVAs to investigate the effects of collection date, habitat location on body length on *T. thermophilum* males, females, and mancas collected from Sedillo Spring and the SIPF between May 1996 and July 1998

Source	DF	Sum of squares	Mean square	*F*-ratio	Prob > *F*
Males					
Model	14	701.33	50.10	14.60	< 0.0001
Error	222	761.64	3.43		
Total	236	1462.97			
Date	10	82.44		2.40	0.0100
Pool	4	574.79		41.88	< 0.0001
Females					
Model	14	196.96	14.07	19.84	< 0.0001
Error	649	460.14	0.71		
Total	663	657.10			
Date	10	37.33		5.27	< 0.0001
Pool	4	153.89		54.26	< 0.0001
Mancas					
Model	14	23.51	1.68	5.48	< 0.0001
Error	216	66.17	0.31		
Total	230	89.68			
Date	10	14.93		4.87	< 0.0001
Pool	4	8.35		6.81	< 0.0001

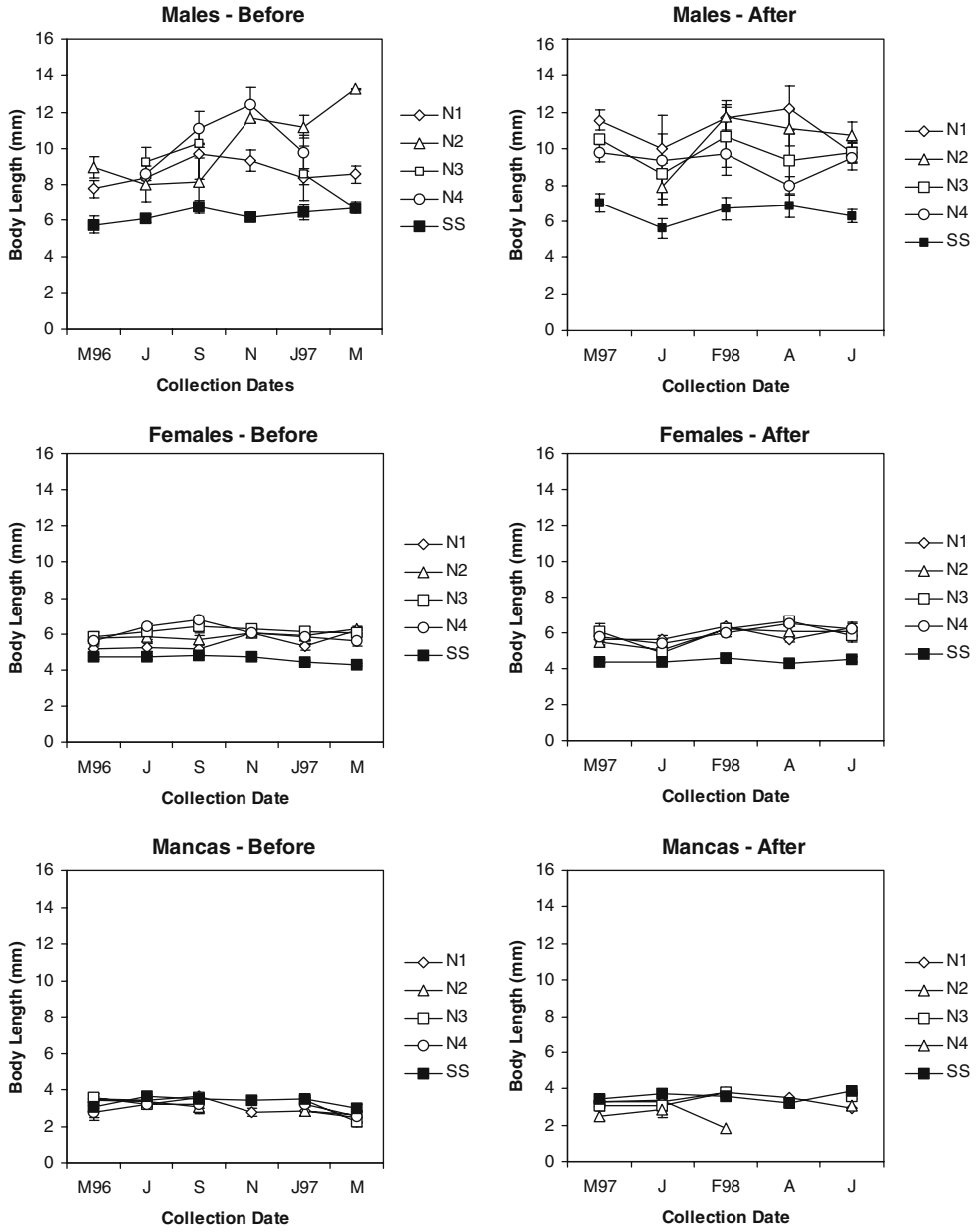

Figure 4. *T. thermophilum* males, females and mancas collected from SS and North pools 1–4 between May 1996 and July 1998, before and after habitat modifications were made to pools N2–N4.

Our 2-level linear analyses revealed significant effects of pool of origin and collection date within each life stage (Table 5; Figure 4), indicating that males, females, and mancas in all populations differed from one another and were variable in body size among collection dates over the interval between 1996 and 1998. Although within-population variation was large for males at each collection date, the effect of this factor on variation in male body length remained significant ($F_{[10,236]} = 2.40$, $P = 0.01$, Table 5).

We considered statistical comparison of isopod body lengths of isopods from natural population with those in captive populations across collection dates unnecessary in this analysis because in all captive populations, at nearly all collection dates,

males and females were significantly larger than males and females in SS. Males in captive populations were 2–3 times larger than SS males (Figure 4). Females in captive subpopulations were consistently larger by half than their ancestral counterparts (Figure 4). When viewed in composite, among all captive populations, variation in male body length was considerably greater than variation in female body length. Although we could not estimate the magnitude of the interaction between collection date and pool for males and females, variation in the body lengths of females, and particularly of males within captive populations, was obvious. We saw no clear patterns in body size variation for mancas (Figure 4).

Of the 15 total regressions of body length on sampling date calculated for males, females, and mancas, we found three significant relationships in the captive and natural subpopulations of *T. thermophilum*. Among males, we found a significant positive relationship in N1 ($F_{1,39} = 11.9$, $P < 0.001$), suggesting that, since no modifications were performed on this habitat in 1997, male body length in this pool continued to increase along a trajectory similar to that which it followed between 1990 and 1996. None of the other regressions were significant for males in the other three captive populations (with $\alpha = 0.003$), or in SS, although the slope of the relationship in N4 was negative ($F_{[1,45]} = 5.63$, $P = 0.022$). This result was consistent with our predictions on the relative strength of selection among pools, and suggests that male body size may have decreased somewhat after vegetation and rock were added to this habitat.

Among females, we found a significant positive relationship between body length and collection date in N1 ($F_{[1,134]} = 13.44$, $P < 0.001$), indicating that in this unmodified habitat, selection on female body size may have been similar in intensity to that imposed between 1990 and 1996. We found no significant relationships between body length and collection date in the other three captive populations, consistent with the results for males. However, we found a significant negative relationship between body length and collection date in SS ($F_{[1,\ 91]} = 11.48$, $P < 0.001$), suggesting either that selection favored small size over this time interval, or as is more likely given our other results suggesting little change in the body lengths of adults in SS over the last 20 years, samples collected after April 1997 contained a disproportionate number of small females. As in the previous analysis, among mancas, no relationships were significant.

We predicted that changes in body lengths of individuals in the captive populations over the collection period would follow the order, N1 > N3 > N2 > N4, consistent with apparent selection intensities due to cannibalism over this interval. The overall pattern of this prediction for males was met although only one of the regressions was significant at $\alpha = 0.003$. N1 males showed significant evidence of selection on body size compared to males in SS ($r^2 = 0.74$, $b = 2.02$, $F_{[1,10]} = 25.6$, $N < 0.001$). Males in N3 were also somewhat larger than males before the selection event in April 1997 ($b = 1.08$). N2 males showed no apparent change in size ($b = 0.05$) and N4 males were actually somewhat smaller than before habitat structure was introduced ($b = -1.17$). No such patterns were observed for females and mancas.

Discussion

That captive subpopulations of *T. thermophilum* diverged genetically from the native population in less than a decade is remarkable. The small genetic differences observed among captive populations, and the tendency toward a pattern of isolation by distance (Figure 2) suggests that sufficient interpool gene flow may minimize detectable genetic divergence within the SIPF. Equally remarkable are our observations that selection favored increased body length in both sexes in four replicate populations of captive isopods, and furthermore, that males were more influenced by selection than females. Rapid evolution of size-associated and other fitness related traits has been observed in captive populations of other species (Heath et al. 2003; Gilligan and Frankham 2003). Our data contribute to the body of literature showing that captive breeding programs can have significant population genetic consequences.

Consistent with the rapid increase in body sizes of individuals from the SIPF are our results suggesting that the high degree of genetic differentiation between natural and captive populations was mainly influenced by two of the AFLP markers identified by molecular genetic analyses (Table 4, Figure 1). Although additional experiments will be required to confirm this observation, our analyses

suggest that these two AFLP marker phenotypes reflect underlying allelic variation at loci that are closely linked to ones that influence body size, and overall, lend additional credence to the use of AFLP analyses in genome scans for identifying genetic markers affected by natural selection (Campbell and Bernatchez 2004).

Before structure was introduced to habitats, captive subpopulations experienced similar selection due to cannibalism, and all captive subpopulations increased in average body size at approximately similar rates between 1990 and 1996. Males may have increased in size faster than females because increased vulnerability of females to predation may have generated a male-biased sex ratio, which intensified selection on males to become large (Ketner et al. 2002). After introduction of habitat structure in the SIPF, especially plant structure, the pattern of divergence changed. Body lengths of isopods in pools N1 and N3 (rocks) continued to increase in size, and did so at greater rates than body length changes occurring in pools N2 (plants) and N4 (plants and rocks). This rate of change was pronounced and statistically significant for N1 males. Evidence of selection was also observed in the body lengths of female isopods after their introduction to SIPF (Figures 3 and 4), although the response of females was less than that of males. Although mancas are presumably affected by predation, we found no evidence that selection influenced the body lengths of immature individuals in any of our analyses.

Our analyses suggested that substrate type may influence cannibalism-induced rates of selection as predicted by Jormalainen and Shuster (1997). That the ability of females and mancas to escape into vegetation may have slowed selection due to cannibalism, as well as the rate of change of female body size, is important for population management in controlled and natural populations. We also note that despite the potential for stronger selection due to cannibalism on mancas and females, a male biased sex ratio, due to the removal of females from the population by cannibalism, combined with sexual selection, due to advantages large males experience during mate guarding, together may have favored large male body size disproportionately compared to females. Further experiments are necessary to explore these differential effects of cannibalism in this species.

Controlled propagation continues to play a major role in the recovery and management of endangered species (Federal Register 2000). The SIPF provides a unique environment for empirical research, and thus stands to contribute important lessons for the propagation and management of this and other threatened populations. The results of this section 6 project (USFWS 1996; Federal Register 2000) corroborate the intent of the Department of Interior's (1998) ''Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act.'' More importantly, our results demonstrate the detail and significance of evolutionary genetic research that can be performed by species-specific conservation management of ''non-charismatic'' invertebrates such as the Socorro Isopod.

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