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Quantitative evidence that both Hsc70 and Hsp70 contribute to thermal adaptation in hybrids of the livebearing fishes *Poeciliopsis*

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Abstract The 70-kilodalton heat shock protein family is composed of both environmentally inducible (Hsp) and constitutively expressed (Hsc) family members. While the role of the constitutively expressed stress proteins in thermotolerance is largely unknown, de novo expression of stress proteins in response to elevated temperatures has been associated with increased thermotolerance in many cell lines, developing embryos and adult organisms. Distinct, hemiclonal hybrids between the livebearing fish species *Poeciliopsis monacha* and *P. lucida* varied in their abilities to survive temperature stress, with survival being greatest when rates of temperature increase to 40°C were slowest and when *P. monacha* genomes were combined with a sympatric *P. lucida* genome. Quantification of Hsp70 under heat shock conditions and Hsc70 under normal physiological conditions indicated that variation in survival among hemiclones was best explained by the combined effects of these two proteins. Similar complex interactions between maternal and paternal genomes and rate of temperature increase were found to underlie patterns of survival, Hsp70 accumulation and Hsc70 abundance. These data suggest that the relationship between Hsps and thermotolerance is more intricate than previously thought and that Hsps contribute to thermal adaptation in these fishes through genetic interactions specific to particular environments.

INTRODUCTION

The induction of heat shock proteins (Hsps) is a genetic response to environmental stress (Lindquist and Craig 1988; Parsells and Lindquist 1993). Typical inducers of this response are pH changes (Whelan and Hightower 1985), heavy metal exposure (Heikkila et al 1982), oxidative stress (Subjeck and Shyy 1986) and elevated temperatures (Lindquist and Craig 1988), all of which disrupt native protein conformation ('proteotoxicity'; Hightower 1991). Hsp70 and other Hsps act as molecular chaperones, preventing improper inter- and intra-molecular interactions involving partially folded proteins. For example, under normal physiological conditions constitutively expressed Hsc70 assists in the proper folding of nascent polypeptides as they emerge from polysomes by preventing aggregation and premature folding (Georgopolous and Welch 1993; Hightower et al 1994). Stress-inducible Hsp70 may rescue denatured proteins from aggregation, help remove damaged proteins and possibly augment Hsc70 functions in stressed cells (Parsells and Lindquist 1992).

Associations between expression of Hsps and transiently acquired resistance to temperature stress have been observed in numerous biological systems (Lindquist and Craig 1988; Parsells and Lindquist 1992) although

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this is not universally the case (Hall 1983; Easton and Spotila 1987; Smith and Yaffe 1991). It is becoming clear that at least two states of thermotolerance acquired by distinct mechanisms exist, one independent of, and another dependent on, de novo Hsp synthesis (Boon-Niermeijer 1986; Laszlo 1988). Investigation of the role of constitutively expressed Hsps in the development of thermotolerance is just beginning.

To augment studies of heat shock isoform diversity in *Poeciliopsis* (White et al 1994; Norris et al 1995), variation in the regulation of Hsp70 and Hsc70 was assayed in four genetically distinct *P. monacha-lucida* hybrids expressing identical Hsp70 and Hsc70 isoforms. Other studies of stress proteins and thermotolerance have emphasized the induction of the response and newly synthesized proteins. Here, the relationships of Hsp70 and Hsc70 to thermotolerance are explored using total abundance of these proteins prior to and over the entire period of stress. In addition, variation among hemiclones in thermotolerance was investigated using assays which measure intrinsic thermotolerance (resistance to acute temperature stress) and acquired thermotolerance (resistance to gradual temperature stress). By combining two distinct hemiclonal *P. monacha* genomes with alternate genomes from homozygous lines of *P. lucida*, it was possible to ascribe variation among hemiclones in these phenotypes to maternal genomes, paternal genomes, experimental conditions and their interactions.

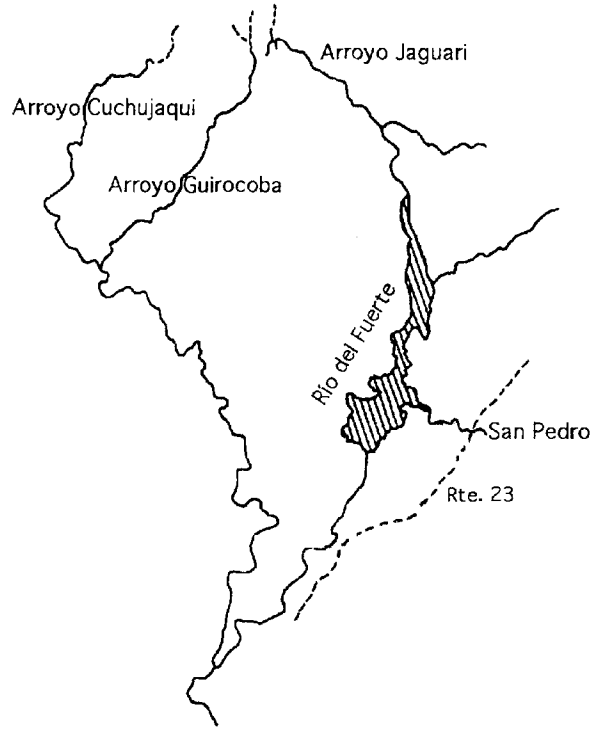

Fig. 1 Collection sites for hemiclonal *Poeciliopsis* and *P. lucida*. Hemiclones VII and VIII were collected from the Arroyo Jaguari. The inbred line S68-4 *P. lucida* was derived from the Arroyo Jaguari population. Inbred M61-31 *P. lucida* was derived from individuals collected from the Río San Pedro.

MATERIALS AND METHODS

Origins and maintenance of fish stocks

Unisexual (all-female) diploid hybrids between *P. monacha* and *P. lucida* reproduce hemiclonally, whereby the paternal *P. lucida* genome is incorporated and expressed in zygotes but is lost prior to oogenesis in adult females (Cimino 1972; Schultz 1989). There is no recombination between maternal and parental genomes. The maternal *P. monacha* genome is inherited clonally, while the paternal genome must be replaced every generation. When mated to a homozygous line of *P. lucida*, all progeny of a hemiclone are genetically identical and differ from other hemiclones mated to the same *P. lucida* line only at maternal loci.

Hemiclones VII and VIII were collected in 1968 and 1970 from a tributary of the Río Fuerte called Arroyo Jaguari near the town of Agua Caliente in northern Mexico (Fig. 1). These are the only *P. monacha-lucida* hybrids found at this locality; they have not been collected at other sites in the 30 years that these fishes have been actively studied. Water temperatures in the arroyo frequently reach 40°C, but vary spatially and seasonally. The fish often swim into hot springs that are abundant in the area where they temporarily expose themselves to water temperatures of 50°C (Schultz et al 1993). Although the Arroyo Jaguari is isolated from the main stream of the Río Fuerte during the dry season, this is not a permanent barrier to movement and does not entirely explain the restricted distributions of these hybrids. Hemiclones VII and VIII are fixed for alternate *P. monacha* alleles at two loci (of 23 examined) distinguishing them genetically; tissue grafts between hemiclones are rejected (Vrijenhoek et al 1978; Angus and Schultz 1979).

Both hemiclones of *P. monacha-lucida* were mated to two homozygous lines of *P. lucida*: one (S68-4 *P. lucida*) derived from a sympatric population and one (M61-31 *P. lucida*) from an allopatric population found in the Río San Pedro (Fig. 1). Henceforth each combination of maternal and paternal genomes will be referred to as VII-S, VIII-S, VII-M and VIII-M. All four hybrids express identical complements of inducible and constitutively expressed Hsp70 isoforms (Norris et al 1995).

Females from each line of *P. lucida* and from each hybrid combination were acclimated 5–7 days at 30°C under a 14L:10D photoperiod at densities equal to, or less than, one fish per gallon of water. They were fed

trout chow twice daily, but were not fed the morning of an experiment. Tank temperatures were regulated with thermostats capable of maintaining temperatures within ± 0.2°C. Tanks were aerated to eliminate temperature stratification and temperature selection by fish.

Heat stress conditions

Fish were placed in plastic containers holding a volume of acclimation tank water equal to 250 ml per fish. This was covered and allowed to cool to 25°C. The water was continuously aerated to maintain maximum O_2 saturation. Stress was initiated approximately 5.5 h into the photoperiod (at about 1100 h).

Stress protocols consisted of placing the plastic container in a water bath and taking the fish from 25°C to 40°C at constant rates of increase (0.15°C/min, 0.3°C/min, 0.5°C/min, or acute stress; henceforth, 0.15, 0.3, 0.5 and acute). At these rates of increase fish spent 100, 50, 30 and 0 min, respectively, between 25°C and 40°C. Fish were held at 40°C for 1 h. Survivors were returned to their acclimation tanks and were monitored for 24–48 h. Different fish were used in each survival experiment and for quantification of Hsp70 and Hsc70.

Overall variation in survival probabilities for every combination of maternal genotype, paternal genotype and rate of temperature increase was assessed using G-tests of homogeneity. To identify significantly different subsets of survival probabilities, a posteriori tests were conducted on these data using the Simultaneous Comparisons Procedure; levels of significance were adjusted accordingly (Sokal and Rohlf 1981).

Analyses of Hsp70 and Hsc70

Hsp70 accumulation was analyzed by repeating survival assays with two individuals per genotype per treatment. At the end of stress, fish were sacrificed by administering an overdose of tricaine (Sigma Chemical Co.). Gill tissue was removed and proteins solubilized in lysis buffer (9.5 M urea, 2% NP-40, 1.6% pH 5–7 ampholytes, 0.4% pH 3.5–10 ampholytes, 5% β-mercaptoethanol, 70 mM Tris HCl pH 7.6, and 1% sodium dodecyl sulfate). Homogenates were agitated briefly and spun in a microfuge for 4 min at room temperature. Supernatants were removed and stored at -70°C.

Tube gels were prepared as described in Norris et al (1995) with the following modification: mixtures of ampholytes (LKB ampholines, Sigma Chemical Co.), were optimized to separate the major inducible and constitutively expressed 70-kDa proteins. A typical mixture was 120 µl pH 5–7 and 30 µl pH 3.5–10 ampholines per 3 ml gel solution. Proteins were separated in the second dimension using SDS-PAGE (9% or 10% resolving gels). Gels were silver-stained according to the manufacturer's protocol with the exception that gels were placed in reducer solution for 30 s and rinsed briefly in distilled water prior to adding the silver solution (Sigma Chemical Co.). This resulted in more reproducible and uniform staining of Hsp70 family proteins.

Silver-stained gels were scanned using a Molecular Dynamics Computing Densitometer (model 300A). Local backgrounds were used in the quantification of major inducible isoforms of Hsp70 (isoform "3"; White et al 1994; Norris et al 1995) and Hsc70 (Fig. 2). The optical density of Hsp70 was normalized to that of Hsc70 to standardize for variation between silver-stained gels in development time and differences in amounts of protein loaded. Levels of Hsc70 are stable, or increase only slightly, under heat shock conditions (L.E. Hightower, personal communication).

The amount of Hsp70 that accumulated in heat-stressed fish was estimated from two-dimensional gels by multiplying the relative optical density (Hsp70 to Hsc70) from stressed fish by the mean nanogram (ng) Hsc70 per milligram (mg) wet weight of gill tissue measured in unstressed fish (see below). Amounts of Hsp70 that accumulated under the four stress treatments were analyzed using a three-way analysis of variance with maternal genotype, paternal genotype and rate of temperature increase as fixed main effects using the Statview software package (Statview 1992). Only statistically significant results are presented. Because the sample sizes for Hsp analysis were small ($n = 2$ individuals per genotype per treatment), significant results represent minimum estimates of genotype and treatment influences on Hsp70 expression.

Direct estimation of the *P. monacha* contribution to Hsc70 abundance in these two hemiclones was not possible because they represent wild genomes and cannot be reconstituted as homozygous, diploid *P. monacha*. An indirect estimate of the maternal contribution was made by measuring the appropriate differences between levels of Hsc70 expressed in acclimatized, unstressed S68-4 *P. lucida*, M61-31 *P. lucida* and VII-M, VIII-M, VII-S and VIII-S.

Abundance of constitutively expressed Hsc70 was measured in acclimated, unstressed fish as follows. Gill tissue was removed from anesthetized fish (four fish per genotype) and mg tissue wet weight was determined. Lysis buffer was immediately added and proteins were processed as described above. These samples were diluted 1:1 with 2X Laemmli sample buffer for one-dimensional SDS-PAGE (Laemmli 1970).

Samples were loaded alongside dilutions of known amounts of purified bovine Hsc70 (kindly provided by Sau-Mei Leung) which served as a standard curve. A separate standard curve was generated for each gel. Gels

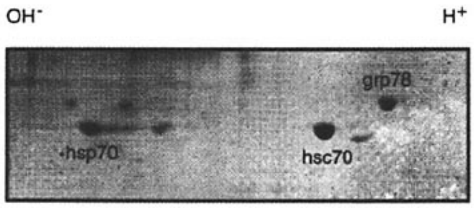

Fig. 2 Two-dimensional electrophoretic separation of inducible Hsp70 (isoform 3), constitutively expressed Hsc70 and Grp78. Proteins were previously identified by radiolabel and immunoblot studies (White et al 1994; Norris et al 1995).

were silver-stained and bovine Hsc70 and sample Hsc70 were quantified using laser densitometry. Total Hsc70 in gill was back-calculated and expressed as ng per mg wet weight of gill tissue. Total quantities of Hsc70 in all four hemiclones and both lines of *P. lucida* were subjected to a one-way analysis of variance using genotype as the main effect; to determine which genotypes expressed similar amounts of Hsc70, all pairwise comparisons among genotypes were made using Fisher's Protected Least Significant Difference procedure for unplanned comparisons (Statview 1992).

RESULTS

Survival of *Poeciliopsis* hemiclones

Four genotypically different hemiclones of *P. monacha-lucida*, when subjected to different rates of temperature increase, had significantly different probabilities of survival (Table 1). Three groups were distinguishable:

- a low survival group composed solely of VII-M (0.3, 0.5, acute stress; outlined by an oval in Table 1)
- an intermediate group composed of genotype VIII-M under acute and 0.5 stress conditions (outlined by a square)
- a high survival group composed of VII-S and VIII-S genotypes under all conditions, VII-M in response to 0.15, and VIII-M in response to 0.15 and 0.3.

Survival was generally higher when both *P. monacha* genomes were combined with the sympatric (S68-4) *P. lucida*, indicating a significant paternal effect. However, differences in survival for all genotypes in response to the 0.15 and 0.3 treatments could not be explained solely by main maternal, paternal and treatment effects. This suggests that a significant interaction among these three variables contributed to the observed differences in survival under these conditions. The survival of VII-M and VIII-M was consistent with earlier studies of stress tolerance in these fish (Bulger and Schultz 1979). S68-4 *P. lucida* was more resistant to thermal stress than M61-31 *P. lucida* under 0.15, but less resistant than any of the hemiclones (data not shown), so increased thermotolerance in the hybrids was not due to greater thermal resistance of the paternal S68-4 *P. lucida* alone.

Hsp70 accumulation

The average amount of inducible Hsp70 accumulated was the same for all hemiclones (data not shown). However, the effect of rate of temperature increase on Hsp70 accumulation was significant (Fig. 3). Genotypes accumulated the most Hsp70 in response to 0.3 and the least in response to acute stress. Levels of Hsp70 accumulated in response to 0.15 and 0.5 were intermediate.

Significant interactions between

1. paternal genotype and treatment
2. paternal genome, maternal genome and treatment on the accumulation of Hsp70 were detected (Fig. 4).

The contribution to total Hsp70 accumulation in the hemiclones made by the paternal × treatment interaction was calculated in the analysis of variance as follows. Abundance of Hsp70 in hemiclones sharing the same *P. lucida* genome and subjected to the same stress conditions was averaged, regardless of differences in *P. monacha* genomes, since by ANOVA there was no significant maternal effect on Hsp70 abundance. These averages were called least squared means and are more appropriate measures of the patterns of paternal contributions to Hsp70 accumulation in the hemiclones than estimating them as one-half the diploid *P. lucida* response. The latter is a measure of the response of a haploid *P. lucida* in the genetic background of *P. lucida* whereas the former is the response when combined with *P. monacha*. These *P. lucida* contributions are not necessarily equivalent. Average contributions (least squared means) of S68-4 *P. lucida* and M61-31 *P. lucida* to total Hsp70 were plotted against rate of temperature increase to visualize the significant paternal × treatment interaction (Fig. 4A). Levels of Hsp70 accumulation under acute heating conditions were similar. However, in S68-4 *P. lucida* amounts increased in response to 0.5 and 0.3 whereas, in M61-31 *P. lucida*, the amounts did not substantially increase until 0.15, the slowest heating rate.

The contribution of S68-4 *P. lucida* to Hsp70 accumulation in the hemiclones increased rapidly under stress: high levels of Hsp70 accumulated in response to acute heating and then amounts of Hsp70 increased further in response to 0.5 (VII-S, VIII-S) and 0.3 (VIII-S), an 'early' response. In contrast, the increase in Hsp70 above amounts produced in response to acute heating occurred only in response to the slower heating rates in the two

Table 1 Survival of hemiclosed VII-M,< VIII-M, VII-S and VII-Sa

	VII		VIII	
	M	S	M	S
0.15	20 / 0	16 / 1	18 / 0	16 / 0
0.3	1 / 20	17 / 1	18 / 1	14 / 0
0.5	2 / 11	14 / 1	34 / 11	14 / 2
acute	1 / 13	19 / 0	20 / 15	27 / 1

[a]Overall heterogeneity in probabilities of survival for the four hemiclones under all rates of temperature increase was significant (GH = 182.03, d.f. = 15.0, $P << 0.001$). The lowest probabilities of survival are circled, intermediate probabilities are in the small square and the remainder have the highest probabilities of survival (threshold X^2 (0.05, 15) = 25.0). The lack of detectable maternal, paternal or treatment main effects in the data represented by outlined numerals suggested the presence of a three-way interaction influencing survival. The proportion is the number of fish surviving/number dead for each treatment.

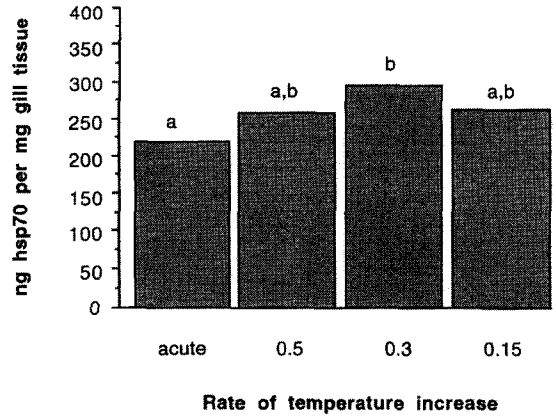

Fig. 3 Average accumulation of Hsp70 by hemiclones in response to four experimental rates of temperature increase. A significant effect of rate of temperature increase on Hsp70 accumulation was detected (F = 3.4, d.f. = 3.0, $P = 0.04$). Similar responses are indicated with the same letter (Fisher's Protected Least Significant Difference procedure, threshold $\alpha = 0.05$).

hemiclones containing M61-31 *P. lucida*, a 'late' response. This occurred in response to 0.3 and 0.15 for VII-M and in response to 0.15 for hemiclone VIII-M.

Generally, patterns of Hsp70 accumulation in hemiclones (Fig. 4B, C) reflected those of their respective paternal genomes (Fig. 4A), with more exaggerated early and late responses. VII-S and VIII-S possessed an 'early' response and survived acute stress in high proportions; hemiclones VII-M and VIII-M possessed a 'late' response and significantly reduced survival (Table 1). However, differences in survival of hemiclones VII-M and VIII-M were distinguishable: VIII-M acquired thermotolerance more quickly than VII-M, despite the fact that VII-M accumulated higher levels of Hsp70 in response to 0.3.

The significant contribution to Hsp70 accumulation by each hemiclonal *P. monacha* genome was estimated as the difference between the paternal × treatment interactions and their respective maternal × paternal × treatment interactions (Fig. 5). Contributions of hemiclonal *P. monacha* genomes VII (Fig. 5A) and VIII (Fig. 5B) to Hsp70 accumulation were different when combined with S68-4 *P. lucida* and M61-31 *P. lucida*, indicating that identical Hsp70 isoforms can be differentially regulated in varying genetic backgrounds. In addition, the patterns of Hsp70 accumulation made by *P. monacha* maternal genome VII are very different from those of genome VIII. Generally, the largest effects on the *P. monacha* contributions were in response to the 0.3 rate of temperature increase.

Abundance of Hsc70

Significant variation in levels of Hsc70 was found among hemiclonal genotypes and both lines of *P. lucida* (Fig. 6). Amounts of Hsc70 in the hemiclones were largely a result of the levels expressed in their respective paternal genomes. The inferred haploid complement from the appropriate line of *P. lucida* accounted for much of the Hsc70 expressed in VII-S, VIII-S and VIII-M, and almost all of that expressed in VII-M. That total Hsc70 decreases when the paternal genome is M rather than S in hybrids with *P. monacha* genome VII but not in hemiclones with *P. monacha* genome VIII indicates a non-additive genetic component to this variation as well. Because hemiclonal genomes cannot be made homozygous, not every genotypic combination was available for analysis. This non-additive effect was marginally significant ($P = 0.0593$) when amounts of Hsc70 were analyzed among hemiclones only and the maternal and paternal contributions could be assessed (data not shown).

DISCUSSION

Thermotolerance and Hsc70 abundance

Survival of hemiclones VII-M and VIII-M increased when the rate of temperature increase to 40°C was slowest, suggesting that thermotolerance is an acquired phenotype in these fishes. For all genotypes, the probability of surviving temperature stress was dependent on the combined effects of maternal genome, paternal genome and rate of temperature increase. Survival was reduced when both hemiclones VII and VIII were mated to the line of *P. lucida* derived from an allopatric population and when rates of temperature increase were rapid. Elevated survival of VII-S and VIII-S was not due solely to the greater resistance of S68-4 *P. lucida*: while this line was more

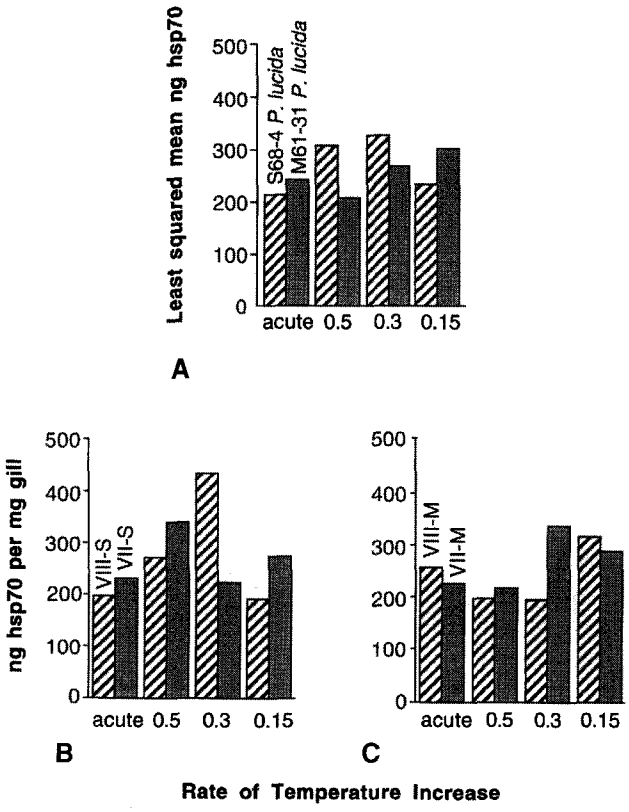

Fig. 4 Plot of the interaction between paternal genome and rate of temperature increase and the influence of this interaction on Hsp70 accumulation (F = 6.4, d.f. = 3.0, $P = 0.0048$) (Panel A). S68-4 *P. lucida* exhibits an 'early' response and M61-31 *P. lucida* a 'late' response (see text). Plots of Hsp70 accumulation in hemiclones (i.e. the three-way interaction) illustrate the early responses of VII-S and VIII-S (Panel B) and the late responses of VII-M and VIII-M (F = 12.056, d.f. = 3.0, $P = 0.0002$) (Panel C). Accumulation of Hsp70 was not detected in the absence of temperature stress.

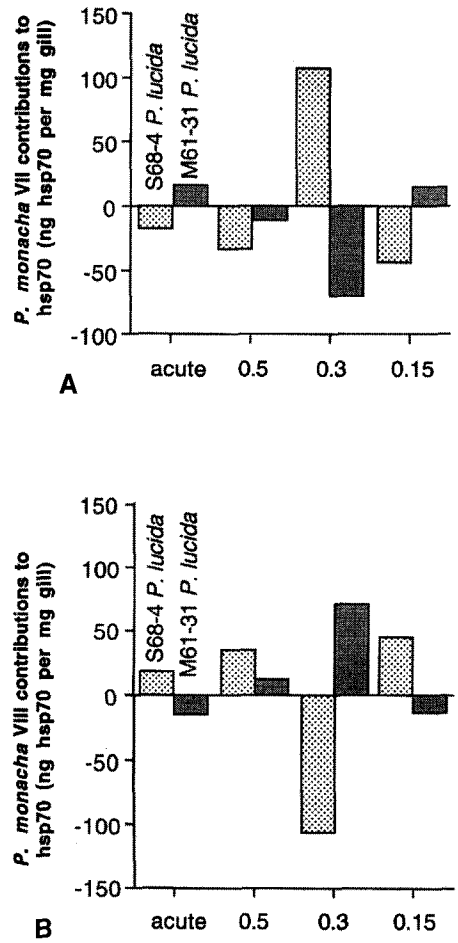

Fig. 5 Estimated contributions of hemiclonal *P. monacha* genomes VII (Panel A) and VIII (Panel B) to Hsp70 accumulation when combined with M61-31 and S68-4 *P. lucida* genomes. Values of Hsp70 are the difference between each *P. lucida* (Fig. 4A) and their respective hemiclones (Fig. 4B, C). Solid bars are the *P. monacha* contributions when combined with M61-31 *P. lucida* and the stippled bars are the *P. monacha* contributions when combined with S68-4 *P. lucida*.

resistant to 0.15 heat stress than M61-31 *P. lucida*, both were significantly more sensitive than any hemiclone (diIorio, unpublished data). Reduced survival of M61-31 *P. lucida* may be due to its inbred nature. However, an extensive survey of breeding histories, another trait with a presumably complex genetic basis, of M61-31 and S68-4 *P. lucida* failed to detect inbreeding depression (Fielding 1992).

Constitutively expressed Hsps have been hypothesized to play a role in stress resistance (Boon-Niermeijer et al 1986; Laszlo 1988; Ulmasov et al 1992; Krebs and Loeschke 1994; Lyashko et al 1994). Increased levels of Hsc70 correlated with increased thermotolerance in species of lizards (Ulmasov et al 1992) and desert-adapted humans (E'vgenev 1995). The heat resistant phenotype of a variant isolated from a mammalian cell line subjected to repeated cycles of heating and recovery was associated with increased levels of Hsc70. Increased expression was due to amplification of the Hsc70 gene (Chen et al 1996). Recent in vitro studies of purified mammalian Hsc70 suggest a mechanism by which a chaperone may contribute to acquired thermotolerance. As temperature increases from about 30°C, Hsc70 undergoes a conformational change which activates its peptide/unfolded protein binding activity (Leung et al 1996). Hemiclones VII-S, VIII-S and VIII-M expressed significantly more Hsc70 than VII-M. Interestingly, VII-M shows lowest survival in response to moderate or acute heat stress. While there appears to be a residual contribution of Hsc70 from *P. monacha* in hemiclone VIII, amounts of Hsc70 in hemiclones VII-M and VII-S can be almost entirely accounted for by the haploid paternal contributions, assuming that our subtraction procedure

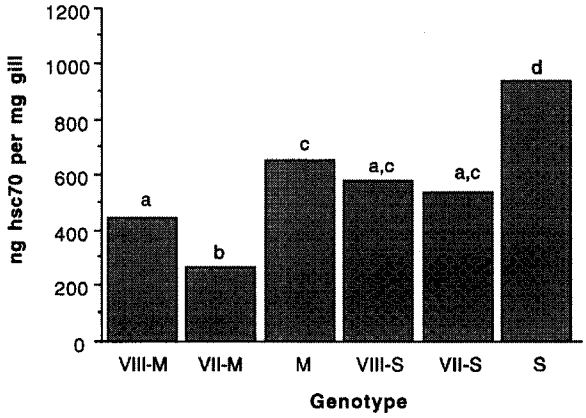

Fig. 6 Levels of Hsc70 expressed gills of acclimated, unstressed hemiclones VII-M, VIII-M, VII-S, VIII-S, M61-31 *P. lucida* and S68-4 *P. lucida*. Genotypes with different letters expressed significantly different amounts of Hsc70, as determined from a posteriori comparisons using Fisher's Protected Least Significant Difference after one-way ANOVA ($\alpha = 0.05$).

accurately separates maternal and paternal contributions. By this analysis, the *P. monacha* Hsc70 contribution in hemiclone VII appears to have been silenced. Whether there has been a silencing mutation in hemiclone VII cannot be determined from this analysis, but null alleles have been found in *P. monacha*-occidentalis hemiclones (Quattro et al 1992). Alternatively, the lack of detectable contribution from the hemiclone VII genome might reflect a generalized incompatibility between the genomes of the two sexual species.

Hemiclones VII-S and VIII-S survived temperature stress better than VII-M (acute, 0.5 and 0.3) and VIII-M (0.5 and acute). In addition VII-S, VIII-S and VIII-M expressed higher levels of Hsc70, which may confer resistance to acute stress in hemiclones by repairing or eliminating damaged proteins before they accumulate to significant amounts. Differences in Hsc70 abundance cannot entirely account for differences in hemiclone survival in response to heat stress. VIII-M expresses amounts of Hsc70 comparable to those found in VII-S and VIII-S, but shows significantly lower survival to 0.5 and acute stress.

Thermotolerance and accumulation of Hsp70

Inferred contributions to Hsp70 accumulation by S68-4 and M61-31 *P. lucida* were significantly different. In homozygous *P. lucida*, S68-4 exhibited an 'early' response, accumulating more Hsp70 in response to rapid rates of temperature increase while M61-31 possessed a 'late' response, accumulating the most Hsp70 only when rates of temperature increase were slow. Replication of the 'early' S68-4 pattern of Hsp70 accumulation in VII-S and VIII-S and of the 'late' M61-31 pattern in VII-M and VIII-M suggests that the significant interaction observed between paternal genotype and rate of temperature increase was due to dominance of the paternal heat shock response, with some maternal epistatic contribution. However, patterns of Hsp70 accumulation did not fully explain the greater survival of VIII-M compared to VII-M in response to 0.3, 0.5 and acute.

The combination of Hsc70 and Hsp70 acting as molecular chaperones better explains the patterns of thermotolerance observed in hemiclones than either protein alone. In these fishes, high levels of Hsc70 and an 'early' heat shock response (VII-S, VIII-S) are associated with high survival; high levels of Hsc70 and a 'late' heat shock response (VIII-M), are associated with reduced survival to acute stress; when reduced levels of Hsc70 are combined with a slow heat shock response, fish (VII-M) succumb to all but the mildest stress conditions. Interactions between Hsc70 and Hsp70 abundance were also correlated with thermotolerance in the tropical *P. gracilis* (Norris et al 1995).

Hsp70 regulation and the evolution of *Poeciliopsis* hybrids

Clonally reproducing organisms are thought to be evolutionary 'dead-ends', due in part to an accumulation and inability to purge deleterious mutations ('Muller's Ratchet'; Muller 1964). Null and deleterious mutations accumulate in clonally inherited *P. monacha* genomes (Leslie and Vrijenhoek 1980; Quattro et al 1992). Nevertheless, hemiclones are ecologically very successful and often outnumber their sexually reproducing counterparts (Schultz 1982); they appear to be evolutionarily long-lived (Quattro et al 1992).

Previous studies of hemiclonal diversity and the ecological success of these all-female lineages have focused solely on variation among clonally inherited *P. monacha* genomes. Extant hemiclones were viewed as the survivors of repeated cycles of hybridization and clonal selection (Schultz 1973; Vrijenhoek 1984; Wetherington and Vrijenhoek 1987). This view suggested that diversity among hemiclones was restricted to a subset of the variation for ecological traits already existing in sexually reproducing *P. monacha*. Further, it suggested that these traits could not vary among identical, hemiclonal *P. monacha* genomes.

Our results suggest that differences among hemiclones are not simply the result of variation among clonally inherited maternal genomes. *P. lucida* can contribute significantly to diversity in hybrid capacities to survive thermal stress by interacting with the *P. monacha* genome in a way that is environmentally dependent. This contribution is consistent in survival data and measures of Hsp70 and Hsc70 abundance, suggesting that Hsps do contribute to

thermotolerance and that hemiclones might be locally adapted to their sexual hosts. These observations reconcile, in part, the prediction of hemiclonal extinction due to Muller's Ratchet and the observation that hemiclonal assemblages are stable through time and may have existed for as much as 300 000 generations (Quattro et al 1992).

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