Monogenic control of iris coloration in the January tetra (Hemigrammus hyanuary Characidae)

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QTL for Pubertal and Seasonality Traits in Male Père David’s × Red Deer Interspecies Hybrids

G. J. C. Goosen, K. G. Dodds, M. L. Tate, and P. F. Fennessy

The unique Père David’s (Elaphurus davidianus) × red deer (Cervus elaphus) backcross hybrid has been used to search for evidence of quantitative trait loci (QTL) for antler pubertal (date and live weight at pedicle initiation) and antler seasonality (date of antler cleaning and casting) traits in temperate species of deer. Analyses using marker information revealed evidence for a QTL for date at pedicle initiation (LOD = 3.7) and live weight at pedicle initiation (LOD = 3.1). These QTL explained 13% and 11% of the phenotypic variance in these traits, respectively.

Many species of deer have evolved as seasonal mammals in response to seasonal variation in their natural environment (Lincoln 1985). Their seasonality is entrained by the changing pattern of daylength (Guinness et al. 1971; Lincoln 1985) and is evident in their highly seasonal patterns of oscillating breeding, feeding, body weight, and moulting, as well as hormone levels (e.g., prolactin, luteinizing hormone, testosterone, growth hormone, and insulin-like growth factor). The seasonal cycle is regulated by changes in melatonin levels, which increase with decreasing daylength and vice versa (Bubenik and Smith 1985; Webster et al. 1991).

In red deer stags the annual pattern of testes growth and regression is also a seasonal phenomenon where decreasing daylength after the summer solstice and increasing testosterone in autumn trigger an accelerated growth pattern that is associated with the breeding season (Barrell et al. 1985; Lincoln 1971b).

The annual seasonal cycle of antler casting and regrowth is largely controlled by circulating plasma testosterone secreted by the testes (Fennessy and Suttie 1985; Suttie et al. 1984). In red deer stags, casting occurs as circulating testosterone levels decline in early spring. Subsequent antler regeneration follows during the period of low testosterone in the spring and early summer (Fennessy et al. 1988; Suttie et al. 1984). The final stage in this annual cycle is antler hardening (essentially conversion to bone and the associated velvet cleaning), which occurs in response to rising testosterone levels in the late summer and autumn so that the stag is in hard antler for the rut (Lincoln 1971b). Velvet cleaning is the process during which the outer antler soft tissue layer dries and effectively peels off, leaving the exposed hard antler.

The antler cycle is preceded by pedicle initiation in the first winter and spring of the stag’s life. The pedicle is the bony protuberance that grows from the skull of the deer from which the first antler grows. Pedicle initiation is a secondary sexual character whose development is associated with the onset of puberty (Lincoln 1971a) and is highly correlated with live weight. A threshold live weight is required irrespective of age or season prior to initiation (Fennessy and Suttie 1985; Meikle et al. 1992). In addition, testosterone administration promotes pedicle development in both prepubertally castrated males and intact females (Fletcher 1978; Jaczewski 1976; Wislocki et al. 1947). The first external signs of antler growth are apparent when the pedicle is about 4–6 cm long (Li and Suttie 1994).

Probably the most accurate indicator of seasonality in males is casting date. Adult Père David’s cast their antlers in June–July and clean their hard antlers in late October–November (Fennessy and Mackintosh 1992). In comparison, typical adult reds cast their antlers in September–October and clean their hard antlers in January–February (Fennessy and Mackintosh 1992). Thus the difference in seasonality between the grand-parental species in antler cycle is on the order of 3 months. The differences in the timing of the antler cycle between F1 (PDxR) and red males is around 57 days, but based on few animals (Fennessy and Mackintosh 1992).

Thus the red deer is a short-day breeder with its reproductive cycle synchronized by a decreasing daylength. In contrast the Père David’s deer (Elaphurus davidianus, PD) is a long-day breeding species (Loudon et al. 1989). Thus the interspecies hybrid is not only unique but also offers an extraordinary opportunity to search for quantitative trait loci (QTL) for pubertal and seasonality traits in deer. Evidence for QTL for gestation length (Goosen et al. 1997) and birth weight (Goosen et al. 1998) has also been documented for these hybrids.

Materials and Methods

Hybrid Generation

F1 hybrids were generated by artificial insemination (AI) of R hinds with PD semen. Backcross hybrid progeny (½ PD/¾ R) were generated by AI of R hinds with F1 hybrid (PDxR) semen (Asher et al. 1988, 1993) and by synchronized natural mating techniques (Fennessy et al. 1991). Semen from five F1 stags was used in a total of 841 laparoscopic intrauterine inseminations. More detailed methods have been described elsewhere (Goosen 1997; Tate et al. 1997). A total of 320 backcross progeny were generated, of which 275 were by AI (240 live at 24 h) with the remainder being produced by synchronized natural mating. During the calving season hinds were monitored daily, newborn calves tagged, and birth weight, gender, and dam recorded.
Table 1. Multiple marker interval mapping maximum likelihood analysis for pubertal and seasonality quantitative trait loci in male Pére David’s × red deer backcross hybrids (PD/R)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marker interval</th>
<th>LOD</th>
<th>Effect</th>
<th>σ%</th>
<th>Linkage group</th>
<th>Marker</th>
<th>Interval (cM)</th>
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<tr>
<td>Antler parameters (date)</td>
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<tr>
<td>Initiation</td>
<td></td>
<td>3.7</td>
<td>21</td>
<td></td>
<td>SPTB—BtaM90</td>
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<td>25.5</td>
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<td>Cleaning</td>
<td></td>
<td>1.7</td>
<td>−5</td>
<td></td>
<td>ADHS—207</td>
<td></td>
<td>1.2</td>
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<tr>
<td>Casting</td>
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<td>1.8</td>
<td>−6</td>
<td></td>
<td>At3—BtaM4311</td>
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<td>7.6</td>
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<td>Live weight (kg) at</td>
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<tr>
<td>Pedicle initiation</td>
<td></td>
<td>7.3</td>
<td>4.5</td>
<td></td>
<td>LALBA—IFNG</td>
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<td>6 months</td>
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<td>2.3</td>
<td>3.4</td>
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<td>CRP—CelJP13</td>
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<td>15 months</td>
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<td>2.0</td>
<td>−3.7</td>
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<td>SOD1—BtaM6438</td>
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<tr>
<td>6 months</td>
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<td>1.2</td>
<td>0.21</td>
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<td>OCAM—COX7A2</td>
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<td>15 months</td>
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<td>2.9</td>
<td>0.33</td>
<td></td>
<td>ANX1like1—GNAT1</td>
<td></td>
<td>24.9</td>
</tr>
</tbody>
</table>

*Highest LOD score is presented irrespective of significance level. Significant linkage LOD > 3.2, suggestive linkage LOD > 1.9.
*Effect of substitution of a red deer allele with a Pére David’s deer allele (PD-R) at the QTL. If the value is negative then substitution is associated with a decrease in the trait.
*Percentage of variance explained by QTL σ% = ((effect/2)/σ²).

Phenotypes

Pedicle development was monitored from weaning every 2 weeks using a technique that measured the height of the pedicle (medial aspect) from the base of the skull, using a steel rule. Pedicle initiation was arbitrarily defined as the date on which the pedicle reached a height of 1.5 cm from the skull (Meikle et al. 1992). Linear regression of pedicle height on date was used to determine the date of pedicle initiation (where it did not coincide with the day of measurement). Antler growth was measured from initiation and throughout the growth period.

Primary antler velvet cleaning was monitored every 3 days from late January and the date of cleaning was defined as the date on which velvet was completely free of soft velvet tissue. Linear regression of degree of cleaning on date was used to determine the date of cleaning (where it did not coincide with the day of measurement). Previous observations of regenerated (or secondary) antler growth indicated it cleaned more quickly than primary antler, giving a more accurate measurement of cleaning date. Thus to more accurately define cleaning date one antler was removed (when the antler reached 20 cm in length) during the phase of active growth using a standard local anesthetic procedure to allow secondary growth. However, the seasonal nature of the antler cycle in some stags meant that removing an antler late in the season would not have resulted in regrowth, so in these cases both sides were allowed to grow naturally. The first choice of measurement used in the analyses was the cleaning date from the secondary antler growth (because it was more accurate); where this was not possible the mean of the two sides was used. For the small number of animals that did not generate secondary antler growth after removal (at 20 cm in length), the one cleaning date was used for analysis.

To define the date of antler casting, stags were monitored every second day during the winter and spring period. Testes diameters at 6 months and 15 months were measured while stags were anesthetized. In all cases the measurement used in analyses was the mean diameter of the left and right testes, except for six animals at 6 months where the only measurable testis was used (i.e., the second testis had not descended into the scrotum).

Genotypes

The hybrid status of all backcross hybrids was confirmed by DNA typing and that of F₁ hybrids by DNA and/or protein testing (Tate et al. 1995). The segregation of up to 250 genetic markers was analyzed in the backcross herd, including restriction fragment length variants (RFLVs), protein variants, and microsatellites. The term “variant” has been used for the fixed differences observed between the species, whereas the word “polymorphism” has been reserved for variation within a species. The species-specific variants used were close to fully informative and on average were 96% informative in backcross offspring in the mapping panels, thus providing a powerful resource for QTL detection. The linkage relationships of the markers were analyzed using MapMaker/EXP using the Kosambi mapping function for all autosomal chromosomes (Tate 1997; Tate et al. 1995). The map was 1240 cm long, with an average spacing between markers of 7.3 cm.

Analysis

Traits were modeled fitting fixed effects of sire and year, while for date and weight at pedicle initiation and the dates of antler cleaning and casting, birth date was fitted as a covariate. In the analyses of testes diameter, weight at the appropriate time was also included.

Linkage analyses investigated differences between backcrosses which inherited either a PD allele or an R allele from the crossbred parent. The interval mapping maximum likelihood package used to test for QTL was MapMaker/QTL (Lander and Botstein 1989).

Results

Multiple Marker Interval Mapping Analysis

Suggestive and significant linkage were defined and calculated (Lander and Kruglyak 1995) using a genome length of 1240 cm and 33 chromosomes and assuming a densely mapped genome. Suggestive and significant linkage using these criteria were LOD = 1.9 and 3.2, respectively.

There was evidence for significant linkage for a pedicle initiation QTL (LOD = 3.7) on linkage group 12 (Table 1 and Figure 1). There were also putative QTL for live weight at pedicle initiation on linkage groups 3, 20, and 31, for 6-month testes diameter on linkage groups 6 and 20, and for 15-month testes diameter on linkage group 24.
Discussion
There was evidence for a pedicle initiation QTL on linkage group 12. Substitution of a red deer allele with a PD allele delayed pedicle initiation significantly. The estimated size of this effect was 21 days using interval mapping. Based on the trends of earlier casting and cleaning in Père David's deer, this was contrary to expectation. The hybrids had a shorter antler-growing season with later pedicle initiation and earlier antler cleaning than reds.

The peak LOD in Figure 1 coincides with a peak in the same region for live weight gain between weaning (at 3 months) and 6-month weight (Goosen et al. 1999). This is perhaps not surprising as pedicle initiation, being a pubertal trait, is a weight-related phenomenon (Fennessy and Suttle 1985; Meikle et al. 1992).

Three regions of the genome (linkage groups 6, 20, and 24) had LOD scores greater than (or equal to) the suggestive linkage for testes diameter, indicating they may be areas of interest for further studies. Since testes diameter at around 6 months could be regarded as indicative of impending puberty, a relationship with pedicle initiation (date or weight) and identification of the same QTL may not have been altogether unexpected. There was evidence for an association between live weight at pedicle initiation and 6-month testes diameter. The same marker location/interval on linkage group 20 was identified as the site for each putative QTL, where the effect of substitution with a PD allele was a higher live weight at initiation and a smaller testes diameter at 6 months.

Similarly there are grounds for expecting that testes diameter at 15 months could be related to date of antler cleaning, but no such relationship was evident. In pedicle diameter at 15 months.

The same marker location/interval on linkage group 20 was identified as the site for each putative QTL, where the effect of substitution with a PD allele was a higher live weight at initiation and a smaller testes diameter at 6 months.


In this article we report the results of experimental crossings designed to answer the question: Do common carp possess one or two GPI-B loci? The ornamental (Koi) variety of common carp was used since most of the European cultured pond carp and wild populations available displayed no or very infrequent variation at GPI-B (Anjum 1995; Kohlmann and Kersten 1999).

Materials and Methods
Koi carp of the broodstock kept at the Institute of Freshwater Ecology and Inland Fisheries, Berlin, were harvested from a rearing pond 4 weeks prior to the expected spawning time. Fish were anesthetized and photographed to allow individual identification. From each specimen a small piece of muscle was taken by biopsy. These samples were analyzed for muscle-specific GPI-B banding patterns. Individuals that showed the three-banded pattern

Figure 1. Examples of GPI banding patterns as observed in the four progeny groups. MO = muscle origin = electrophoresis starting point. Positions of allelic products and between loci heterodimers are marked on the right. Genotypes at the three GPI loci are given on the bottom. Of relevance for the present study is primarily the whole cathodal part of the gel showing the three different GPI-B phenotypes: one strong, slowly migrating band (lane 1 representing the aa homozygote), three bands (lanes 2 and 3 representing the ab heterozygote), and one strong, rapidly migrating band (lanes 4 and 5 representing the bb homozygote). Variation at the GPI-A2 locus used to test for random sampling of fish is visualized in the upper area of the anodal part of the gel. Homozygotes aa (lanes 1 and 2) are characterized by one fast band (third band from top), heterozygotes ab (lanes 3 and 4) display three bands (fourth to sixth band from top), and homozygotes bb (lane 5) show one slow band (third band from top). Number and position of GPI-A1/GPI-B1 and GPI-A2/GPI-B2 heterodimers were used to confirm the genotyping of the GFA2* and GFB2* loci. The remaining bands without annotations represent additional heterodimers of minor importance for the interpretation of GPI banding patterns.
of heterozygotes (see lanes 2 and 3 from the left in Figure 1) were selected for experimental crossings.

Four females and four males were individually mated to produce four independent full-sib progeny groups. Each progeny group was incubated and reared in a separate aquarium. At an age of approximately 3 months, 100 specimens from each group were sampled randomly and sacrificed. Small pieces of muscle were dissected from the dorsal part of the fish, shock frozen at −20°C, and stored at −84°C until analysis. The muscle samples were prepared for electrophoresis by homogenizing them with a Tris-HCl sample buffer, pH 7.0 (Ferguson 1980). Then the extracts were subjected to horizontal starch-gel electrophoresis according to the method described by Vuorinen (1984) using the discontinuous lithium hydroxide-boric acid buffer, pH 8.1 (Ridgway et al. 1970). Staining of the GPI banding patterns (Figure 1) were selected for experimental crossings. Four females and four males were individually mated to produce four independent full-sib progeny groups. Each progeny group was incubated and reared in a separate aquarium. At an age of approximately 3 months, 100 specimens from each group were sampled randomly and sacrificed. Small pieces of muscle were dissected from the dorsal part of the fish, shock frozen at −20°C, and stored at −84°C until analysis. The muscle samples were prepared for electrophoresis by homogenizing them with a Tris-HCl sample buffer, pH 7.0 (Ferguson 1980). Then the extracts were subjected to horizontal starch-gel electrophoresis according to the method described by Vuorinen (1984) using the discontinuous lithium hydroxide-boric acid buffer, pH 8.1 (Ridgway et al. 1970). Staining of the GPI banding patterns followed the procedures of Shaw and Prasad (1970). Locus and allele nomenclature was according to Shaklee et al. (1990).

The segregation of the alleles ‘a(100)’ and ‘b(85)’ of the GPI-A2 locus was used to test for random sampling of fish. Three genetic models were examined for their ability to explain the GPI-B inheritance: (1) one locus segregating for two alleles a and b; (2) two loci, one monomorphic and one segregating for two alleles a and b; (3) two loci, both segregating for two alleles a and b. Since only individuals with three-band ed GPI-B patterns were selected for propagation the following homozygous parental genotypes could be excluded from these models: aa and bb (model 1), aa,aa (model 2), aa,aa and bb,bb (model 3). All remaining possible mating combinations were considered to calculate the expected genotypic and phenotypic ratios of progenies. For models (2) and (3) the expected phenotypic ratios could be summarized to two and six categories, respectively (Table 1). These ratios were compared to the observed number of progenies with different GPI-B phenotypes within the four experimental groups. After the genetic control of the GPI-B phenotypes was clarified, frequencies of composite GPI-A2’,GPI-B’ genotypes were calculated to examine whether these two genes are located on different chromosomes or not. All statistics were done using the chi-squared test of the SPSS for MS Windows version 6.1 software package.

### Results

In skeletal muscle extracts the enzymatic products coded both by GPI-A’ and GPI-B’ loci were active, with the majority of those moving cathodally and coded by GPI-B’. All between-loci heterodimers were formed of the polypeptides coded by GPI-A1’, GPI-A2’, and GPI-B’ loci; the heterodimers moved anodally and were helpful in deciphering both GPI-A’ and GPI-B’ polymorphism (Figure 1).

The segregation of the alleles ‘a(100)’ and ‘b(85)’ of the GPI-A2’ locus demonstrated random sampling within each progeny group (Table 2). The expected genotypic ratios were 1aa:2ab:1bb in groups 1, 2, and 4 and 1aa:1ab in group 3. The largest but still insignificant deviation from Mendelian expectations was observed in group 3. This group also contained a specimen of genotype bb, which could not originate from the parents of that group if mutations are excluded. More probably, some unintentional mixing must have happened in this group.

The observed banding patterns (Figure 1) and phenotypic ratios (Table 1) for GPI-B fitted only to model 1 by chi-squared analysis (Table 3). In each of the four progeny groups the observed ratios were in accordance with the expected ratio of 1:2:1 for a single locus with two alleles.

After verifying that common carp GPI-B is coded by only one locus, the hypothesis of an independent, dihybrid inheritance of the two loci GPI-A2’ and GPI-B’ could be tested. The parental composite genotypes GPI-A2’,GPI-B’ were ab,ab × ab,ab for progeny groups 1, 2, and 4, resulting in an expected ratio of progeny genotypes of 1aa,aa:2aa,ab:1aa,bb:2ab,aa:4ab,ab:2ab,bb. In all four groups the observed ratios were in agreement with the expected ones (Table 4). Again, the largest but still insignificant deviation from Mendelian expectations was observed in group 3.

### Discussion

Subsequent to the original tetraploidalization event there had to be two duplicate GPI-B’ genes; in some fish species of tetraploid origin they remained duplicate (for instance, in salmonids; Allendorf and Thorgaard 1984), whereas in some others, like in the cyprinid Carassius auratus, one of the two duplicate genes has been silenced in their expression in the process.

The possibility of a secondary loss of one of the duplicated GPI-B* loci in common carp has been neither proved nor rejected until the present study. The hypothesis on the existence of duplicate GPI-B1* and GPI-B2* loci was supported by the fact that among more than 460 common carp individuals sampled from 12 stocks and examined by Anjum (1995), there were more than 80 GPI-B*-100/-180 heterozygotes but no GPI-B*-180/-180 variant homozygotes. In this case at least one of the examined stocks was against Hardy–Weinberg expectations if there is a single locus only. The second locus must have been silenced in the course of time after the tetraploidization event. The dihybrid inheritance model was supported by the fact that GPI-B in common carp is coded by two loci.

The results of the experimental crossings reported in this article clearly show that GPI-B in common carp is coded by one locus only. The second locus must have been silenced in the course of time after the tetraploidization event. The dihybrid inheritance of loci GPI-A2* and GPI-B* demonstrates that both loci are located on different chromosomes.

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Karyotype Identity of Two Subspecies of Eld’s Deer [Cervus eldi] — C. e. eldi, C. e. thamin, and C. e. siamensis — C. e. siamensis is considered to be particularly endangered following its disappearance from a major portion of its original range. The only captive breeding population of this subspecies is in the zoological parks at the Paris Museum of Natural History. Taking into account its low effective population size (N_e = 7) and the increasing levels of inbreeding, the continued breeding of this “micropopulation” in isolation from closely related subspecies and in particular from C. e. thamin, which is much more common in zoos as well as in the nature, is questioned. That an initial step in determining if crosses between these subspecies could be performed without risk of outbreeding depression due, in part, to gross differences in their karyotypes, a comparative chromosome banding analysis (RBG-bands) of C. e. siamensis and C. e. thamin was undertaken. No chromosomal differences were identified between the taxa at the level of resolution obtained. The study suggests that, at least from a karyotypic perspective, no obvious differences delimit the two subspecies, and hybridization between endangered C. e. siamensis and C. e. thamin is not likely to lead to impaired fertility in hybrid animals.

In terms of conservation status, 11 of the 17 species currently recognized in the subfamily Cervinae are considered as threat-
Figure 1. RBG-banded karyotype of male C. e. siamensis.

ened to a varying degree (Grubb 1993). Two of these, Cervus eldi and C. nippon, are usually cited among those for which there is the most concern (IUCN 1996). Eld’s deer (C. eldi), the subject of the present study, comprises three subspecies, C. e. eldi, C. e. thamin, and C. e. siamensis (Groves 1995; Whitehead 1993), although a fourth C. e. hainanus, is sometimes recognized (Song 1993).

As recently as the turn of this century C. e. siamensis was recorded as having a range that extended through the entire Indo-Chinese subregion (Cheminaud 1939; Millet 1930). Presently the size of the only natural population known to remain in Vietnam is estimated at approximately 100 animals (Decoux 1994). The only captive population of C. e. siamensis is held in the zoological parks at the Paris Museum of Natural History. It comprises seven animals that are characterized by relatively high inbreeding coefficients varying between 0.28 and 0.41 (Decoux 1994). This clearly questions the wisdom of continued breeding within this population and underscores the need to identify potential breeding stock that would have to be drawn from one of the other closely related subspecies; the most likely candidate, based on prevalence of zoo populations and numbers in the wild, is C. e. thamin.

As has been demonstrated, cytogenetic analysis permits the detection of separate chromosomal rearrangements which may have the potential to reduce fertility (or in extreme cases induce hybrid sterility) and increase mortality in animal populations (Dyrendahl and Gustavsson 1979; Kingswood et al. 1994; 1998a,b; Ryder et al. 1989; Vassart et al. 1994), a detailed comparative banding study of C. e. siamensis and C. e. thamin is a prerequisite to any recovery program that would involve interbreeding between the two subspecies. Although the G-banded karyotype of C. e. thamin has been previously published (Neitzel 1987), that of C. e. siamensis is unknown. As a first step toward defining management units within C. eldi and to evaluate the potential risks of the intersubspecific hybridization, we present herein the results of a comparative R-banding chromosome analysis of C. e. siamensis and C. e. thamin. We establish complete correspondence of their R-banding patterns, thus permitting the discounting of one of the risk factors responsible for outbreeding depression.

Materials and Methods

Chromosome preparations were obtained from cell cultures established from skin biopsies of eight C. e. siamensis (five males and two females and one aborted male fetus) and a single female of C. e. thamin; all animals are housed at the Zoological Park of Paris and La Haute Touche. R banding (RBG; ISCN 1995) was done following Hayes et al. (1991). A minimum of 30 metaphases were analyzed for each specimen.

Results and Discussion

The karyotype of C. e. siamensis, described here for the first time, comprises 6 pairs of large submetacentric and 22 pairs of large to middle-size acrocentric autosomes, and two acrocentric gonosomes which are, respectively, the largest (X) and smallest (Y) elements in the genome (Figure 1). Side-by-side comparisons show complete correspondence of the C. e. siamensis and C. e. thamin R bands (Figure 2). The 2N = 58 and NFa = 70 found in our C. e. thamin study animal is identical to that reported for this taxon by Neitzel (1987). Among the eight C. e. siamensis and one C. e. thamin specimen karyotyped, no visible heteromorphism or polymorphism was detected.

While conservation at the species level has conventionally been the focus of captive breeding programs, there has been an increasing awareness of the need to conserve at the population level, since it is
Here that responses to environmental challenges occur. Ralls and Ballou (1986) propose considering the species as the management unit if no outbreeding depression is observed in the offspring of crosses between distinct populations. At the same time Ryder (1986) proposes to establish the "evolutionary significant unit" (ESU) as a management unit, based on geographical, morphological, behavioral, and genetic aspects of the population. Although the ESU was introduced to help conservation biologists determine appropriate taxonomic subunits to guide conservation efforts, it was criticized by Moritz (1994), who instead advanced a concept of "management units" (MUs). The two terms are currently used to clarify conservation units. The debate over what exactly constitutes a "significant" unit has not yet been resolved. If one follows Moritz (1994), "ESUs should be reciprocally monophyletic for mitochondrial DNA alleles and show significant divergence of allele frequencies at nuclear loci." In this case the ESU is relevant to long-term conservation strategies. However, within the ESU there may be an important genetic substructure in which there is such low levels of gene flow between demes that they could be considered as functionally independent units. These are what Moritz (1994) called management units (MUs) and these are regarded as being logical units for short-term management considerations.

If we came back to the case of C. eldi and its subspecies, the situation is further complicated by the absence of comprehensive genetic data for these taxa. By this reason we do not know if the subspecies generally recognized are the real taxonomic units. For example, Deuve (1972) states that there are no accurate pheno-

typic or other criteria that permit their unambiguous discrimination; moreover, he described C. e. siamensis itself as morphologically polytypic.

According to Groves (1995), breeding across subspecies could cause the loss of their genetic, ecological, and behavioral particularities, and this loss is definitive. However, the converse is also true. Failure to rectify the deleterious effects of inbreeding depression can also precipitate the loss of biodiversity. While our data show that C. e. siamensis and C. e. thamin share identical R-banding patterns, this does not mean that there are no genetic differences between them. However, the existence of fertile hybrids between C. e. siamensis and C. e. thamin (Decoux 1994) suggest that the effects of outbreeding depression, if any, will be low.

Within the Eld’s deer conservation program it appears extremely important to continue molecular genetic investigations (now in progress) in order to test the validity of the subspecies and to determine what entity may be considered as an MU (Moritz 1994). In the short term, however, the study does raise the possibility of introducing new breeding stock of C. e. thamin, especially from the wild, to supplement the C. e. siamensis herd presently in captivity at the zoological parks of the Paris Museum of Natural History.

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References


Ryder OA, Kumamoto AT, Durrant BS, and Benirschke E, 1994. Cytogenetic offspring. Coloration within each progeny group was scored at 10 weeks following initiation of feeding. Mottled coloration was observed in none of the progeny at this time. The phenotypes observed (palomino, albino, and/or wild type) and their proportions within progeny groups indicated that the mottled female was originally heterozygous for albinism. The fish apparently became mosaic for this trait following mutation of the wild-type allele at the albinism locus within a cell(s) early in embryonic development. Curiously, at approximately 6 months after initiation of feeding, mottled coloration became apparent in 2 fish from among 25 progeny of the cross to the golden male. No change in phenotype was noted at this time in 9 gynogenetic progeny nor in 68 progeny from the cross to the albino male. Apparently additional mutations and/or other genetic and regulatory processes affecting coloration came into play during juvenile development of these latter two fish.

Among a group of rainbow trout being reared at Casta Line Trout Farms, Goshen, VA, an individual with a unique mottled coloration was noted. This fish had large patches of skin of either yellow or dark (wild-type) coloration, and darkly pigmented eyes (Figure 1). The owner of the farm was interested to know if this trait was heritable and whether fish with the mottled coloration might have particular economic value for sportfishing.

We hypothesized that the mottled rainbow trout was mosaic at the golden locus. A female of similar mottled skin coloration and darkly pigmented eyes, pictured in Willers (1991), was the fish from which the West Virginia Centennial Golden rainbow trout strain was derived (Beall 1963; Clark 1970; Wright 1972). Wright (1972) described the nature of golden as being a single-locus, partially dominant autosomal trait. In the homozygous condition, golden (G’G) rainbow trout completely lack melanophores, resulting in appearance of an underlying bright golden-yellow coloration. Heterozygosity for golden (G’G) is associated with a significantly reduced number of melanophores relative to wild-type individuals (GG), and a brownish-yellow coloration. These fish are referred to as palomino rainbow trout. Both goldens and palominos possess normal dark eye pigmentation.

The mottled fish was reared for 3 years to sexual maturity for subsequent progeny testing. Eggs from this female were fertilized with sperm of rainbow trout males of various phenotypes. In addition, diploid gynogenetic progeny were produced. The objectives of these tests were to determine the genetic identity of the mottled rainbow trout, and whether the mottled coloration was a heritable trait.

**Methods**

At 3 years of age, the female rainbow trout with mottled yellow and dark skin pigmentation and dark eyes was manually stripped of its eggs. The eggs were subduced into several groups for production of five different types of progeny. Three groups of approximately 100 eggs each were fertilized with sperm from one of three males of wild-type coloration, obtained the same day from Erwin National Fish Hatchery, Erwin, TN. Two groups of approximately 300 eggs each were fertilized with sperm from a golden rainbow trout or an albino rainbow trout, both available at Casta Line Trout Farms. Development in another two groups of approximately 300 eggs each was activated with ultraviolet (UV)-irradiated sperm from one of the wild-type males, followed by thermal shock to induce second polar body retention for the production of diploid gynogenetic progeny (Chourrout 1982; Palti et al. 1997; Scheerer and Thorgaard 1983). Prior to thermal shocking, approximately 50 eggs were removed from each of the latter two groups to provide haploid control embryos.

The eggs were incubated in separate boxes within a vertical tray incubator. Water temperature averaged approximately 12°C. One week after hatching the fry were transferred to separate screen-sided buckets suspended within a common rearing trough. Progeny from the three crosses to wild-type males were pooled at this time, as were the two groups of gynogenetic fry. Following initiation of feeding the fish

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**Mottled Coloration in a Rainbow Trout Is Associated with Mosaicism for Albinism**

P. F. Galbreath and B. P. Plemmons

Within a group of rainbow trout at a commercial farm, a single individual was noted for its mottled yellow and dark skin pigmentation. This female fish was reared to sexual maturity and sublots of its eggs were crossed to rainbow trout males from golden, albino, and wild-type (dark-pigmented) strains. Development in other eggs was activated to produce diploid gynogenetic offspring. Coloration within each progeny group was scored at 10 weeks following initiation of feeding. Mottled coloration was observed in none of the progeny at this time. The phenotypes observed (palomino, albino, and/or wild type) and their proportions within progeny groups indicated that the mottled female was originally heterozygous for albinism. The fish apparently became mosaic for this trait following mutation of the wild-type allele at the albinism locus within a cell(s) early in embryonic development. Curiously, at approximately 6 months after initiation of feeding, mottled coloration became apparent in 2 fish from among 25 progeny of the cross to the golden male. No change in phenotype was noted at this time in 9 gynogenetic progeny nor in 68 progeny from the cross to the albino male. Apparently additional mutations and/or other genetic and regulatory processes affecting coloration came into play during juvenile development of these latter two fish.

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We hypothesized that the mottled rainbow trout was mosaic at the golden locus. A female of similar mottled skin coloration and darkly pigmented eyes, pictured in Willers (1991), was the fish from which the West Virginia Centennial Golden rainbow trout strain was derived (Beall 1963; Clark 1970; Wright 1972). Wright (1972) described the nature of golden as being a single-locus, partially dominant autosomal trait. In the homozygous condition, golden (G’G) rainbow trout completely lack melanophores, resulting in appearance of an underlying bright golden-yellow coloration. Heterozygosity for golden (G’G) is associated with a significantly reduced number of melanophores relative to wild-type individuals (GG), and a brownish-yellow coloration. These fish are referred to as palomino rainbow trout. Both goldens and palominos possess normal dark eye pigmentation.

The mottled fish was reared for 3 years to sexual maturity for subsequent progeny testing. Eggs from this female were fertilized with sperm of rainbow trout males of various phenotypes. In addition, diploid gynogenetic progeny were produced. The objectives of these tests were to determine the genetic identity of the mottled rainbow trout, and whether the mottled coloration was a heritable trait.

**Methods**

At 3 years of age, the female rainbow trout with mottled yellow and dark skin pigmentation and dark eyes was manually stripped of its eggs. The eggs were subduced into several groups for production of five different types of progeny. Three groups of approximately 100 eggs each were fertilized with sperm from one of three males of wild-type coloration, obtained the same day from Erwin National Fish Hatchery, Erwin, TN. Two groups of approximately 300 eggs each were fertilized with sperm from a golden rainbow trout or an albino rainbow trout, both available at Casta Line Trout Farms. Development in another two groups of approximately 300 eggs each was activated with ultraviolet (UV)-irradiated sperm from one of the wild-type males, followed by thermal shock to induce second polar body retention for the production of diploid gynogenetic progeny (Chourrout 1982; Palti et al. 1997; Scheerer and Thorgaard 1983). Prior to thermal shocking, approximately 50 eggs were removed from each of the latter two groups to provide haploid control embryos.

The eggs were incubated in separate boxes within a vertical tray incubator. Water temperature averaged approximately 12°C. One week after hatching the fry were transferred to separate screen-sided buckets suspended within a common rearing trough. Progeny from the three crosses to wild-type males were pooled at this time, as were the two groups of gynogenetic fry. Following initiation of feeding the fish...
Predicted ratios are based on results from the cross with an albino male which indicated that prior to meiosis 84% \((X)\) of the oocytes were homozygous for albinism and the remainder \((1 - X)\) of the oocytes were heterozygous. G\(\)G = golden; G\(\)g = palomino; GG = wild type, normal dark pigmentation.

AA and aa = wild type; aa = albino.

were reared for 10 weeks, after which the number and proportion of fry within progeny groups was calculated for each coloration phenotype—dark (wild type), yellow with dark eyes (palomino or golden), or yellow with red eyes (albino). No attempt was made to discern palomino from golden coloration, as we found that the distinction is insufficiently certain in fish of this size and age. The phenotypic ratios observed and predicted phenotypic ratios (number per phenotype/total number of progeny) and predicted (in parentheses) ratios among progeny from crosses of the mosaic yellow and dark mottled female rainbow trout with male golden rainbow trout and albino rainbow trout.

### Table 1. Formulas for predicting phenotypic ratios and observed (number per phenotype/total number of progeny) and predicted\(^*\) (in parentheses) ratios among progeny from crosses of the mosaic yellow and dark mottled female rainbow trout with male golden rainbow trout and albino rainbow trout.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotype and phenotype of the male</th>
<th>Formulas for predicting phenotypic ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Palomino</td>
</tr>
<tr>
<td>GG/AA, G/G/AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mosaic for golden)</td>
<td>GG/AA wild type</td>
<td>(X/2 + 1 - X) (X/2)</td>
</tr>
<tr>
<td></td>
<td>GG/aa albino</td>
<td>(X/2 + 1 - X) (X/2)</td>
</tr>
<tr>
<td></td>
<td>G’G/AA golden</td>
<td>(X/2 + 1 - X) (X/2)</td>
</tr>
<tr>
<td>GG/Aa, GG/aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mosaic for albinism)</td>
<td>GG/AA wild type</td>
<td>(1) (1 - X/2) ((1 - X)/2 + X)</td>
</tr>
<tr>
<td></td>
<td>GG/aa albino</td>
<td>(1) (1 - X/2) ((1 - X)/2 + X)</td>
</tr>
<tr>
<td></td>
<td>G’G/AA golden</td>
<td>(1) (1 - X/2) ((1 - X)/2 + X)</td>
</tr>
<tr>
<td>GG/Aa, GG/aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mosaic for albinism)</td>
<td>GG/AA wild type</td>
<td>&gt;200/&gt;200 (1.0)</td>
</tr>
<tr>
<td></td>
<td>GG/aa albino</td>
<td>19/240 (0.08) 221/240 (0.92)</td>
</tr>
<tr>
<td></td>
<td>G’G/AA golden</td>
<td>220/220 (1.0)</td>
</tr>
</tbody>
</table>

\(^*\) X equals the proportion of premeiotic oocytes possessing the mutated G\(\)G or aa genotype, and \(X - 1\) equals the proportion of oocytes with the original GG or Aa genotype.

\(^*\) Predicted ratios are based on results from the cross with an albino male which indicated that prior to meiosis 84% \((X)\) of the oocytes were homozygous for albinism and 16% \((1 - X)\) were heterozygous.

### Table 2. Formulas for predicting phenotypic ratios and observed (number per phenotype/total number of progeny) and predicted\(^*\) (in parentheses) ratios for gynogenetic progeny from the mosaic yellow and dark mottled female rainbow trout in the cases of 100% and 0% crossing-over for the golden locus and the albinus locus.

<table>
<thead>
<tr>
<th>Hypothesized genotype of the mottled female</th>
<th>With 100% or with 0% exchange</th>
<th>Formulas for predicting phenotypic ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG/AA, G/G/AA</td>
<td></td>
<td>Wild type</td>
</tr>
<tr>
<td>(mosaic for golden)</td>
<td>100%</td>
<td>(1 - X) ((1 - X)/2) ((1 - X)/2 + X)</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>(1 - X) ((1 - X)/2) ((1 - X)/2 + X)</td>
</tr>
<tr>
<td>GG/Aa, GG/aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mosaic for albinism)</td>
<td>100%</td>
<td>(5/41 = 0.12) (36/41 = 0.88)</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>(0.16) (0.84)</td>
</tr>
</tbody>
</table>

\(^*\) X = the proportion of premeiotic oocytes possessing the mutated G\(\)G or aa genotype; \(X - 1\) = the proportion of oocytes with the original GG or Aa genotype.

\(^*\) Predicted ratios are based on results from the cross with an albino male which indicated that prior to meiosis 84% \((X)\) of the oocytes were homozygous for albinism and 16% \((1 - X)\) were heterozygous.

G\(\)G = golden; G\(\)g = palomino; GG = wild type, normal dark pigmentation.

AA and aa = wild type; aa = albino.

were then compared to those expected under the hypothesis of mosaicism for golden. Formulas for calculating predicted ratios are presented in Table 1 for the different crosses and in Table 2 for the gynogenetic progeny. Predictions in the latter were calculated for both 100% and 0% exchange between homologous chromosomes during crossing over in meiosis I.

### Results

The observed proportions of fish per phenotype within each progeny group are also provided in Tables 1 and 2. Fry from the crosses with wild-type males accidentally died shortly before 10 weeks following initiation of feeding. However, it was recorded that of the more than 200 fish, all were of wild-type coloration. The fry produced in the two haploid gynogenetic control groups all died prior to or shortly after hatch, confirming the effectiveness of the UV irradiation treatment and that all survivors among the thermal shocked fish were diploid gynogens.

A portion of the fish within each progeny group were kept for continued rearing. Beginning at approximately 6 months, mottled coloration became progressively apparent in 2 fry from among 25 progeny of the cross to the golden male. No change in phenotype was noted at this time in 9 remaining gynogenetic progeny and 68 progeny from the cross to the albino male.

### Discussion

Phenotypic ratios predicted according to our initial hypothesis of mosaicism in the mottled female for a single golden allele (G\(\)G, GG) did not agree with the ratios observed in the progeny tests. The unexpected appearance of albinos among the progeny from the cross with an albino male and among the gynogenetic progeny led to the alternative hypothesis that the mottled female was mosaic for albinism (Aa, aa). Formulas for predicted phenotypic ratios under this hypothesis are also presented in Tables 1 and 2. Albinism in rainbow trout, as in other fish species (Kirpichnikov 1981; Tave 1993), is associated with yellow skin pigmentation and red eyes. The lack of pigmentation is associated with a simple recessive autosomal trait involving a mutation which precludes melanin production (Bridges and von Limbach 1972; Kirpichnikov 1981; Tave 1993). The observed phenotypic ratios in our progeny tests agree well with the alternative hypothesis.
Under this hypothesis, the female was originally heterozygous for albinism (Aa). The mosaic condition was likely the result of a mutation in the single “A” allele to “a” at the albinism locus within a cell(s) early in embryonic development. This hypothesis would have been more predictable when it was remarked later that albinos have occasionally been observed among fish of the commercial strain from which the mosaic female was obtained (Plemmons BP, personal observation).

Dobosz et al. (1999) characterized a double-locus inheritance scheme for yellow coloration in a Polish strain of rainbow trout which differs from that for golden and albinism. This strain also originated from of a fish with mottled skin pigmentation (Dobosz et al. 1999; Maliszewski 1987). However, results in the present tests conform well to the previously described schemes for golden and albinism.

In predicting coloration ratios for gynogenetic progeny, calculations in Table 2 were made for the cases of both 100% and 0% exchange of alleles during crossing over between homologous chromosomes during meiosis I. Crossing over in trout chromosomes is apparently obligatory and occurs only once per chromosome arm such that gynogenetic progeny will be virtually 100% heterozygous for terminally located loci (Allendorf et al. 1986; Thorgaard et al. 1983). Thorgaard et al. (1995) demonstrated that the golden locus is tightly linked to the centromere and that exchange of alleles during crossing over does not occur. In contrast, they demonstrated that the locus for albinism in five of six albino rainbow trout strains was apparently telomeric; exchange of alleles occurred nearly 100% of the time. In our progeny test of the mottled female with an albino male, 8% of the 240 progeny were darkly pigmented. The proportion (1 – χ) of oocytes in the female mosaics which were heterozygous (Aa) upon entering meiosis would therefore be approximately 2 × 0.08 = 0.16 (Table 1). Using this figure, the number of the 41 diplont gynogenetic progeny that would be wild type when exchange during crossing over occurred 100% and 0% of the time was predicted to be seven and three, respectively. The observed number (five) was intermediate and the results could not be used to confirm the percent exchange at this locus during crossing over.

Rainbow trout with mottled skin pigmentation have been observed in a variety of strains, albeit rarely (Beall 1963; Galbreath PF, personal observation; Hinshaw JM, personal communication; Maliszewski 1987; Parsons JE, personal communication; Wright 1972). This odd color pattern appears to be caused by mosaicism, in this case for albinism and in other cases for the golden locus. In both situations, the phenotype is similar in general appearance and the trait is not directly inheritable. Whether or not mottled coloration of the two fish that appeared after 6 months of age involved a postzygotic mutation of the golden or albino gene is unknown. Also, it is unclear what other genetic and developmental mechanisms may have come into play such that the coloration pattern was not expressed until later in the juvenile stage. To help answer these questions, our intention is to rear these two fish until sexual maturity for subsequent progeny testing.

Brief Communications

Does Fitness Erode in the Absence of Selection? An Experimental Test with Tribolium

A. Łomnicki and M. Jasiński

In the absence of natural selection, average fitness in the population is expected to decline due to the accumulation of deleterious mutations. Replicate populations of flour beetles (Tribolium confusum) were maintained for 22 generations in the virtual absence of selection (random mating, favorable environment, excess of food, and mortality of only 3%). Larva-to-adult survivorship rates were similar in the stock population and selection-free populations. In contrast, starvation resistance of adult beetles from selection-free populations was significantly reduced (by more than 2% per generation). When tested in the favorable environment, beetles in one selection-free population had significantly slower development and smaller sizes of females than beetles from the stock population. Since such changes in these fitness components are usually maladaptive, they indicate possible erosion of fitness under relaxed selection at the rate of 0.1–0.2% per generation. No fitness erosion was detectable in the second selection-free population.

The fitness of organisms may be viewed as a compromise between natural selection which tends to raise fitness and several factors that continually erode it (Burt 1995; Fisher 1958). The characterization of the evolutionary dynamics of a population requires the knowledge of how fragile this compromise is, that is, the ease with which the dominance of the eroding factors would lead to maladaptation and a subsequent decline in fitness. The erosion of fitness may be caused, for example, by accumulation of deleterious mutations, genetic drift, or temporal and spatial changes in the quality of the environment. What happens if natural selection, the main
“cleansing agent” of evolution, is absent or drastically reduced?

Reduced selection should allow the accumulation of mutations, usually with mild deleterious effects (Lynch et al. 1999). One can therefore expect that reduced selection should, after several generations of mutation accumulation, bring about a reduction in average individual fitness (Muller 1950). Recent studies have found detectable negative effects of accumulated mutations on various fitness components in Escherichia coli (Kibota and Lynch 1996), Arabidopsis thaliana (Drake et al. 1998), Caenorhabditis elegans (Keightley and Caballero 1997; Vassilieva and Lynch 1999), Daphnia pulex (Lynch et al. 1998), and Drosophila melanogaster (Fry et al. 1999; Shabalina et al. 1997). Eventually, such contamination of the genome may lead to the extinction of small populations (Lynch et al. 1995), or to a high mutation load, even in large populations (Konrashov 1995).

In this study we have attempted to determine the impact of eliminating selective pressure on the components of fitness, through purely demographic means, in laboratory populations of the flour beetle (Tribolium confusum). We have designed a favorable environment with an excess of food and no competition, no larval cannibalism, and random (i.e., phenotype-independent) choice of individuals for mating. The changes occurring over 22 generations in populations maintained under absent or relaxed selection were assessed relative to the original source population, representing the state prior to the evolutionary experiment.

Materials and Methods

Flour beetles of the genus Tribolium are convenient organisms for selection-reduction experiments: their eggs can be easily isolated from media and their individual contribution to the next generation can be controlled (see, e.g., Korzeniak and Jasieński 1990). Prereproductive mortality can be considerably reduced in Tribolium for which the optimal rearing conditions are well established (Sonleitner and Guthrie 1991). The T. confusum beetles used in this experiment were taken from a stock maintained for more than 20 years at the Jagiellonian University (see, e.g., Jasieński et al. 1988). All experimental populations were kept under standard laboratory conditions.

Initially, 25 mated females were allowed to lay eggs for 4 days. From each female, 4 eggs were chosen at random to start a population which was kept in the favorable environment, in the absence of selection. Each egg was placed separately in a glass vial filled with 2 g of medium. After 18–21 days, vials were inspected (or re-inspected in the next 3 to 4 days if the larvae have not pupated). Pupae were weighed to within 0.001 mg, then sexed, and returned to the same vials where they were subsequently left undisturbed for several weeks. The weight measurements were omitted in generations 15, 16, 18, 20, and 21. Twenty five parental pairs were chosen randomly to establish the next generation. This procedure was designed to minimize prereproductive mortality and ensure low variation in individual reproductive contributions. The second replicate pair of populations (lineage 2) was set up identically a week later. Populations were established as described above in each of the 22 generations.

The stock-derived population originated from individuals (30 males and 30 females) that were sampled as pupae in the stock culture at the time when the experiment entered generation 21 and reared for one generation in the favorable environment. In generation 22, growth, development rate, survivorship, and starvation resistance of beetles from unselected populations were compared in the favorable environment with the characteristics of individuals from the stock-derived population.

The egg-hatching success and larva-to-adult survivorship were assessed in selection-free populations. Mortality during the larval, pupal and adult stages was low. Consequently, we analyzed the results by combining all three sources of mortality. Comparisons of the stock-derived cultures with unselected populations from the 22nd generation were carried out in a two-way analysis of variance, with treatments and sex as fixed factors. Following all analyses of variance, specific hypotheses were further tested with pairwise a priori contrasts of treatment means, with the level of significance for the family of tests kept at 0.05. The significance for individual contrasts and for tests of effects in the analyses of variance was determined by the sequential Bonferroni procedure (Rice 1989). Starvation resistance of adult beetles was tested by placing 40–60 individuals in small beakers and monitoring their survival. Mortality rates were compared using the Kolmogorov–Smirnov test.

Results

Larva-to-adult survivorship was very high (about 97%) in the selection-free populations. In addition, approximately 18% of the eggs did not hatch (as assessed in generations 12, 14, 17, 19, and 22 in the favorable environment; Lomnicki A, unpublished data), as they were infertile, unfertilized or larvae that died before hatching. These estimates of early mortality may be combined to yield a maximum estimate of total opportunity for selection of 21% in the selection-free populations.

In the selection-free population, mean pupal weights were 3.19 mg (SE = 0.015; n = 370) for females and 2.82 mg (SE = 0.012; n = 403) for males. The number of eggs laid by the females is known for generations 11, 13, 16, 18, and 21. The average 4-day fecundity of females for all generations pooled together was 49.8 eggs (SE = 0.77; n = 228) for the selection-free population.

After 21 generations in favorable environment, lineage 2 was characterized by significantly longer development time in males and females than the stock population (Tables 1 and 2). In females, this prolonged developmental period was associated with a significant decrease of body mass compared to the stock population. Both differences may be expressed relative to the stock population as declines of fitness per generation of mutation accumulation. The declines were 0.13% and 0.18% in the case of female and male pupation times, respectively, and 0.22% in the case of female pupa body mass. In contrast, no significant changes in development time or body size were recorded in the selection-free population of lineage 1 compared to the stock population.

Stock-derived adults had significantly higher starvation resistance than adults from the selection-free treatment. Selection-free beetles survived on the average for 4.9 and 6.2 days (in lineages 1 and 2, respectively), while the stock-derived beetles survived for 10.6 and 11.5 days (in lineages 1 and 2, respectively). Differences between the survivorship curves were significant in both lineages (Figure 1; Kolmogorov–Smirnov test, lineage 1: D = 0.8389, P < 0.001; lineage 2: D = 0.7965, P < 0.001). The mean decline in starvation resistance was 50.0% in the selection-free lineages, yielding an estimate of 2.3% decline per generation.

Discussion

The main question asked in this study is whether relaxation of natural selection oc-
occuring during several consecutive generations of living in favorable conditions might have a negative impact on the average fitness of individuals in a randomly mating population. While one is unlikely to encounter such a situation in natural conditions, fitness erosion may be relevant in captive populations (Frankham and Loebel 1992) or in germplasm storage programs (Schoen et al. 1998). In these cases, selection intensity may be greatly reduced by favorable environmental conditions combined with, for example, equalization of progeny number. The modern human population has also been invoked as a possible candidate for fitness erosion due to weakened selection (see e.g. Crow 1993, 1997; Muller 1950; Takahata 1993).

The term "selection-free", or "unselect- ed", as used in this article, means that natural and artificial selection are absent. It differs therefore from the usual usage in quantitative genetics, where the term refers to a population currently not experiencing artificial selection on any particular trait. In such a population natural selection is usually still operating, as determined by the laboratory conditions and alleles selectively disadvantageous in nature may become advantageous in captivity (Frankham and Loebel 1992). A clear distinction between directional selection and some unspecified pressure of natural selection is important also because the latter may be more effective than the former in preventing the erosion of fitness, even though the variables selected for in directional selection may be closely associated with fitness (see also Frankham and Robertson 1980). In this study, selection intensity was reduced to a minimum, set, for example, by uncontrollable and potentially selective mortality during the embryonic stages.

Is the deterioration affecting only minor adaptations, with negligible effects on the overall fitness of the individual, or can it also affect major components of fitness? The erosion of fitness may, in fact, not be noticeable when the deterioration of adaptations impacts only the fine-tuned and specialized characteristics, whose contribution to fitness was rather small or only occasionally important. Highly specialized adaptations are likely to be destroyed by even small pressures of mutation or drift. The background of generalized adaptations may frequently provide sufficient support, and fitness may show signs of erosion only when those generalized adaptations start deteriorating in the extended absence of selection.

There is a growing body of evidence that fitness does decline due to mutation accumulation, but the estimates vary by two orders of magnitude among different species (for a discussion of the reasons for such variation among species and studies see Lynch and Walsh 1998 and Vas-sileva and Lynch 1999). The decline in the number of surviving offspring from a D. melanogaster female was 0.2% per generation (over 30 generations) when tested under favorable conditions, and 10 times greater when tested under a competitive regime (Shabalina et al. 1997). Fecundity, however, declined by only 0.003% per gen-

### Table 1. Mean body mass of pupae and pupation times in the stock control population and in selection-free populations tested in the favorable environment

<table>
<thead>
<tr>
<th></th>
<th>Lineage 1</th>
<th>Lineage 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selection-free</td>
<td>Stock</td>
</tr>
<tr>
<td><strong>Females (N)</strong></td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Pupation time (days)</td>
<td></td>
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<tr>
<td>Mean</td>
<td>22.5</td>
<td>22.9</td>
</tr>
<tr>
<td>SE</td>
<td>0.40</td>
<td>0.31</td>
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<tr>
<td>Body size (mg)</td>
<td>3.128</td>
<td>3.255</td>
</tr>
<tr>
<td>SE</td>
<td>0.0633</td>
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</tr>
<tr>
<td><strong>Males (N)</strong></td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>Pupation time (days)</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>23.2</td>
<td>22.3</td>
</tr>
<tr>
<td>SE</td>
<td>0.42</td>
<td>0.32</td>
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<tr>
<td>Body size (mg)</td>
<td>2.723</td>
<td>2.781</td>
</tr>
<tr>
<td>SE</td>
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<td>0.0479</td>
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</tbody>
</table>

Statistically significant results are indicated by * at the $P \leq 0.05$ level and ** at the $P \leq 0.01$ level. See Table 2 for the descriptions of the statistical tests conducted.

### Table 2. Analysis of variance table for body mass at pupation and pupation time in the favorable environment: comparisons of selection-free populations with their stock control populations

<table>
<thead>
<tr>
<th></th>
<th>Lineage 1</th>
<th>Lineage 2</th>
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<td>Residual</td>
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<td>Wilks' $\lambda$</td>
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<td>142</td>
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<tr>
<td>Sex</td>
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<td>142</td>
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<tr>
<td>Population * sex</td>
<td>2</td>
<td>142</td>
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</tbody>
</table>

Significant effects are marked as bold.
ever, in the absence or relaxation of selection in the laboratory, the selective re-
selection pressure imposed by the condition for an increased pupal weight
increased) and fitness. For example, in one
selection pressure. One must therefore cor-
correlated with those subject to weak se-
strong selection pressure are negatively
occasionally lead to an increase of fitness
mutation-accumulation lines. Theor Appl Genet 56:57–64.
References
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1999). If inbreeding depression were pro-
nounced in this study, some of the reces-
sive mutations would likely be eliminated
during the embryonic development. How-
ever, the female fertility recorded in this
study is not lower than the typical esti-
mates of fertility reported by Sokoloff (1974) for noninbred strains of this spe-
cies.
Irrespective of whether the deteriora-
ion one might expect a backward slippage in
response to the decay of link-
age disequilibrium (Travis and Mueller
1989). This effect might confound the anal-
ysis of the response of the population to
the relaxation of selection. Therefore, the
net result of eliminating selection pressure
(an increase, decrease or no noticeable
change in fitness) cannot be predicted,
larva will pupate late and small, with neg-
If the larva is growing slowly, then the
larva will pupate late and small, with neg-
itive consequences for survivorship and
adult reproductive success (Sokoloff
1974). This result which was observed in
the selection-free population (lineage 2)
when compared with the stock may there-
fore indicate a case of fitness erosion. The
magnitude of such erosion (about 0.2%
per generation) is similar to the values ob-
tained from the studies on Drosophila (Fry
et al. 1999; Shabalina et al. 1997).
A relaxation of selection pressure may
occasionally lead to an increase of fitness
(Lynch et al. 1998). This effect may occur
if the components of fitness subject to
strong selection pressure are negatively
correlated with those subject to weak se-
lection pressure. One must therefore cor-
correctly identify relationships between
the expression of the trait (increased or de-
creased) and fitness. For example, in one
study there was a decline in population fit-
ness with increasing pupal weight in
Tribolium, although 100 generations of selec-
tion for an increased pupal weight
resulted in a shift of the mean equivalent
to 17 standard deviations from the base
population (Enfeldt 1980). If the original
stock culture was under some artificial se-
lection pressure imposed by the condi-
tions in the laboratory, the selective re-
ponse in this population might have been
due in part to linkage disequilibrium aris-
ing between epistatically-linked loci. How-
ever, in the absence or relaxation of selec-
tion pressure imposed by the condi-
tions in the laboratory, the selective re-
spone in this population might have been
due in part to linkage disequilibrium aris-
ing between epistatically-linked loci. How-
ever, in the absence or relaxation of selec-
tion pressure imposed by the condi-
tions in the laboratory, the selective re-
spone in this population might have been
due in part to linkage disequilibrium aris-
ning between epistatically-linked loci. How-
ever, in the absence or relaxation of selec-

Monogenic Control of Iris Coloration in the January Tetra (Hemigrammus hyanuary Characidae)

J. S. Frankel

The January tetra (Hemigrammus hyanuary Durbin) exhibits two eye color phenotypes. These have a silver iris, which is characteristic of the species, and a green color variant. Segregation patterns observed in the progenies from 12 different crosses support an hypothesis for the monogenic inheritance of iris coloration in this species.

Fishes in the teleost genus Hemigrammus (Characidae) exhibit a wide variety of body colorations and marking patterns (Axelrod and Vorderwinkler 1995; Mills 1993; Vevers 1980). The January tetra (Hemigrammus hyanuary Durbin), so named because of its discovery in Lake January of the Amazon basin, is one of the more recently introduced characins in North America. Natural populations of H. hyanuary exhibit two distinct iris coloration phenotypes. Characteristically this species displays an iridescent greenish-yellow stripe along the flanks with a silver-colored iris of the eye. Indeed, the species is often marketed as the “green neon” due to its distinctive body and stripe coloration. A phenotypic variant of H. hyanuary exhibits a “grass-green”-colored iris (Vevers 1980), along with the same body and stripe coloration as silver-eyed fishes. Iris coloration is easily discerned in this species, with green-eyed fishes clearly distinguishable from their silver-eyed counterparts. As a result of ongoing studies in my laboratory on several species of characins (Supriharti 1996; Supriharti and Frankel 1998) I had the opportunity to investigate the inheritance patterns for iris coloration in the January tetra.

Materials and Methods

Healthy adults of H. hyanuary, exhibiting either a silver iris or the grass-green color variant, were obtained from Mid-Atlantic Distributors, Inc. (Springfield, VA), and maintained in 20 gal holding tanks at 26°C. Male and female H. hyanuary exhibiting the silver and green phenotypes were selected at random from stock specimens, placed in separate 20 gal holding tanks, and allowed to develop until sexually mature. All progeny for this study were obtained from artificial fertilizations as described for the cyprinid fishes Brachydanio rerio and B. altolineatus (Frankel and Hart 1977; Hart and Messina 1972) with water conditions as described for natural matings of Hemigrammus (i.e., 5° of hardness or less with a pH of 7.0 at 26°C) (Axelrod and Vorderwinkler 1995). Parents of both silver iris (S) and green iris (G) phenotypes, along with selected F1 progeny, were used in a series of 17 crosses (Table 1; crosses 10–26). Embryos from all crosses were incubated at 26°C in 250 ml fingerbowls containing tank water. Dead or developmentally arrested embryos were removed daily. Immediately after hatching, fry from each cross were placed in holding tanks and allowed to develop until their iris color phenotype could be determined. Phenotypic data of all progeny were recorded and subjected to chi-square analysis.

Results and Discussion

Table 1 presents the probable genotypes, observed phenotypic numbers, expected ratios, and probability of fit for H. hyanuary analyzed for the mode of inheritance of iris coloration. Parental fishes and progeny from all crosses displayed either the silver or green iris phenotype. Silver iris parental females S-1, S-II, and S-III, and males S-1 and S-2 were scored as homozygous for a dominant allele, as all crosses involving these individuals resulted in silver progeny (crosses 1–5, 10–14). In addition, a silver-eyed female (S-IV) and a male (S-3) were scored as heterozygous for this allele (see below). Parents exhibiting the green iris phenotype (G-I, G-II, and G-III females and G-I, G-2, and G-3 males) were scored as homozygous recessives, as all crosses between these individuals consistently bred true (crosses 6–9). Reciprocal crosses between silver (S) and green (G) homozygous parents always resulted in silver fry (matings 10–14). Complete dominance of an allele encoding the silver-eyed phenotype was confirmed by backcrosses of green-eyed parents (G-H and G-III females, and G-1 and G-2 males) with their silver-eyed progeny, and by crosses of the S-IV and S-3 fishes with green-eyed parental G-I and G-3 (crosses 15–18, and...
19 and 20, respectively). Each of these crosses resulted in a satisfactory fit to a silver:1 green ratio. In addition, offspring from F1 \times F1 crosses resulted in a satisfactory fit to a 1:0 ratio.

### Table 1. Probable genotypes (PG), observed phenotypic numbers, expected ratios, degrees of freedom (df), chi-square values ($\chi^2$), and probability of fit ($P$) for crosses among silver-eyed and green-eyed Hemigrammus bynnanuri

<table>
<thead>
<tr>
<th>Cross no.</th>
<th>Parental genotypes</th>
<th>Phenotypic numbers</th>
<th>Expected ratio</th>
<th>df</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S-I (AA) x S-I (AA)</td>
<td>24</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S-II (AA) x S-II (AA)</td>
<td>15</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>S-III (AA) x S-III (AA)</td>
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<td>0:1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S-I (AA) x S-II (AA)</td>
<td>14</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S-III (AA) x S-I (AA)</td>
<td>10</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>75</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>G-I (aa) x G-I (aa)</td>
<td>0</td>
<td>1:0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>G-II (aa) x G-II (aa)</td>
<td>0</td>
<td>20:1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>G-III (aa) x G-III (aa)</td>
<td>0</td>
<td>19:1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Pooled</td>
<td>56</td>
<td>1:0</td>
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</tr>
<tr>
<td>9</td>
<td>S-I (AA) x S-I (AA)</td>
<td>22</td>
<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>S-II (AA) x S-II (AA)</td>
<td>18</td>
<td>0:1</td>
<td></td>
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</tr>
<tr>
<td>11</td>
<td>S-III (aa) x S-III (aa)</td>
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<td>0:1</td>
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<tr>
<td>12</td>
<td>G-I (aa) x G-I (aa)</td>
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<td>1:0</td>
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<td>13</td>
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<td>0:1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
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<td>0:1</td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>G-I (aa) x F-III (Aa)</td>
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<td>G-II (aa) x F-III (Aa)</td>
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<td>.70–.70</td>
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<td>1:1</td>
<td>1</td>
<td>0.667</td>
<td>.50–.30</td>
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<td>18</td>
<td>F-II (Aa) x G-II (aa)</td>
<td>14</td>
<td>1:1</td>
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<td>0.290</td>
<td>.70–.50</td>
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<td>19</td>
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<td>12</td>
<td>1:1</td>
<td>1</td>
<td>0.182</td>
<td>.70–.50</td>
</tr>
<tr>
<td>20</td>
<td>G-I (aa) x S-III (aa)</td>
<td>15</td>
<td>1:1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>1:1</td>
<td>1</td>
<td>1.521</td>
<td>.95–.95</td>
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<td></td>
<td>Pooled</td>
<td>77</td>
<td>1:1</td>
<td>1</td>
<td>1.464</td>
<td>.95–.90</td>
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<tr>
<td>21</td>
<td>F-I (Aa) x F-I (Aa)</td>
<td>20</td>
<td>3:1</td>
<td>1</td>
<td>0.889</td>
<td>.50–.30</td>
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<tr>
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<td>F-II (Aa) x F-II (Aa)</td>
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<td>1</td>
<td>1.514</td>
<td>.30–.20</td>
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<td>F-III (Aa) x F-III (Aa)</td>
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<td>3:1</td>
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<td>0.800</td>
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<td>0.889</td>
<td>.50–.30</td>
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<tr>
<td>25</td>
<td>F-II (Aa) x F-III (Aa)</td>
<td>17</td>
<td>3:1</td>
<td>1</td>
<td>0.223</td>
<td>.70–.50</td>
</tr>
<tr>
<td>26</td>
<td>F-II (Aa) x F-III (Aa)</td>
<td>22</td>
<td>3:1</td>
<td>1</td>
<td>1.283</td>
<td>.30–.20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>78</td>
<td>3:1</td>
<td>1</td>
<td>4.858</td>
<td>.70–.50</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>110</td>
<td>3:1</td>
<td>1</td>
<td>4.398</td>
<td>.50–.30</td>
</tr>
</tbody>
</table>

$^a$ S designates silver iris parentals; G designates green iris parentals; F designates F1, silver iris offspring.

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The presence of highly repetitive DNA sequences in Japanese quail (Coturnix coturnix japonica) was identified from genomic DNA digested with restriction endonuclease, BglII. Sequence analysis of three different-size clones revealed the presence of a tandem array of a GC-rich 41 bp repeated element. This sequence was localized by fluorescence in situ hybridization (FISH) primarily to microchromosomes of Japanese quail (2n = 78); approximately 50 of the 66 microchromosomes showed positive signals, although hybridization signals were also detected on chromosomes 4 and W. This satellite DNA did not cross-hybridize with genomic DNA of chicken (Gallus gallus) and Chinese painted quail (Exocliton chinesis) under moderately stringent conditions, suggesting that this class of repetitive DNA sequences was species specific and fairly divergent in Galliformes species.

A novel satellite DNA sequence of Japanese quail (Coturnix coturnix japonica) was isolated from genomic DNA digested with restriction endonuclease, BglII. Sequence analysis of three different-size clones revealed the presence of a tandem array of a GC-rich 41 bp repeated element. This sequence was localized by fluorescence in situ hybridization (FISH) primarily to microchromosomes of Japanese quail (2n = 78); approximately 50 of the 66 microchromosomes showed positive signals, although hybridization signals were also detected on chromosomes 4 and W. This satellite DNA did not cross-hybridize with genomic DNA of chicken (Gallus gallus) and Chinese painted quail (Exocliton chinesis) under moderately stringent conditions, suggesting that this class of repetitive DNA sequences was species specific and fairly divergent in Galliformes species.

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**References**


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clones. These satellite DNAs consisted of 41 bp tandem repeated elements. The polymorphic sites in the consensus repetitive sequences of Japanese quail with that of chicken and turkey (Matzke et al. 1990, 1992).

Several species-specific tandem repeat DNA sequences have been isolated and localized to W chromosomes or microchromosomes of the chicken and turkey (Kodama et al. 1987; Matzke et al. 1990, 1992; Saitoh et al. 1991; Tone et al. 1984). In this study we cloned a novel satellite DNA sequence from Japanese quail. Here we report the molecular characterization of this satellite DNA sequence, which is species specific and primarily present in microchromosomes of Japanese quail.

Materials and Methods

Molecular Cloning of Satellite DNA

Genomic DNA was extracted from the liver of a female Japanese quail using standard techniques (Sambrook et al. 1989). Satellite DNAs are often observed as satellite bands when eukaryotic genomic DNA is digested with appropriate restriction endonucleases and electrophoresed through agarose gel. Genomic DNA of the female Japanese quail was digested with 20 restriction endonucleases and electrophoresed through agarose gel. Genomic DNA of the female Japanese quail was digested with 20 restriction endonucleases—Bgl II, BglI, ClaI, DraI, EcoRI, EcoRV, HindIII, HpaII, KpnI, NotI, PvuII, SacI, ScaI, SalI, Smal, Stul, XbaI, and Xhol—size fractionated by 2% agarose gel electrophoresis, and stained with ethidium bromide. Bgl II produced the brightest and clearest satellite bands of the 20 enzymes. The satellite DNA bands of Bgl II were eluted from the gel and cloned into plasmid pZErO-2 (Invitrogen). The satellite DNA fragments inserted in pZErO-2 were sequenced by ABI PRISM Dye Primer Cycle Sequencing Kit with M13 forward (-21) and reverse primers, using a model ABI 373S DNA sequencer (Perkin-Elmer).

Southern Blot Hybridization

Genomic DNAs of the three different species, Japanese quail, chicken, and Chinese painted quail (Excallatoria chinensis), were digested with restriction endonucleases and fractionated in 2% agarose gel by horizontal gel electrophoresis. The DNA fragments were transferred onto Hybond-N+ nylon membrane (Amersham) and then the filter was probed with a 192 bp fragment labeled with [α-32P]dCTP by random priming method. Hybridization was carried out overnight at 37°C in 50% formamide, 5 × SSPE, 5 × Denhardt’s, 0.2% SDS, and 100 μg/ml denatured salmon sperm DNA. The membrane was washed at 37°C in 0.1 × SSC and exposed to X-ray film.

Cell Culture and Chromosome Preparation

Japanese quail lymphocytes were cultured for chromosome preparation following the method for mouse spleen lymphocytes with slight modification (Matsuda and Chapman 1995). Lymphocytes were isolated from the spleen of adult female Japanese quail and transferred into culture flasks containing TC199 medium supplemented with 20% fetal calf serum, 3 μg/ml concanavalin A (type IV-S, Sigma), 10 μg/ml lipopolysaccharide (Sigma), 2% HA15 (Murex), and 50 mM mercaptoethanol, and were incubated at 39°C in a humidified atmosphere of 5% CO₂ in air. Mitogen-stimulated lymphocyte cultures were synchronized by thymidine block, and BrdU (25 μg/ml) was incorporated during the late replication stage for replication R-band staining after release from excessive thymidine. R-bands were obtained by exposure of chromosome slides to ultraviolet (UV) light after staining with Hoechst 33258.

Fluorescence In Situ Hybridization

Probe DNAs were labeled with biotin-16-dUTP using a nick translation kit (Roche Diagnostics) and ethanol-precipitated with salmon sperm DNA and E. coli tRNA. Hybridization and detection of fluorescence signals were performed following Matsuda and Chapman (1995). After incubation with fluoresceinated avidin (Vector), the slides were stained with 0.75 g/ml propidium iodide. Fluorescence images were observed by an Olympus BX-60 fluorescence microscope with Olympus filter sets U-MWIB (excitation at 470–490 nm), U-MSWG (480–550 nm), and U-MWU (330–385 nm), and photographed with Kodak Ektachrome ISO 100 films.

Results

Molecular Cloning and DNA Sequence Analysis

Several satellite DNA bands were revealed by agarose gel electrophoresis of Bgl II-digested genomic DNA (Figure 1A). Three different-size DNA bands marked in Figure 1A (about 80–200 bp) were isolated from the gel, cloned into plasmid pZErO-2, and then sequenced. The insert sizes of these clones were 82, 123, and 192 bp (Figure 1B). The nucleotide sequence data of each clone has been entered into the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession numbers AB035968–AB035970. The Bgl II satellite DNA fragments of Japanese quail consisted of 41 bp tandem repeat elements with a few substitutions, and the 41 bp consensus sequence showed high GC content (58–61%). Hairpin loop structures were not observed for this satellite DNA sequence.

DNA Blot Analysis

To examine the abundance of the satellite DNA sequences in Galliformes species, Southern hybridization was performed with genomic DNAs of female Japanese quail, chickens (G. gallus), and Chinese painted quail (E. chinensis) using the 192 bp fragment as a probe. As shown in Figure 2, Japanese quail produced intense hybridization signals, but no signals were found in the other two species, suggesting...
that no homologous sequences exist in the chicken and Chinese painted quail genomes.

Chromosomal Distribution of the Satellite DNA Sequences

FISH analysis was applied to localize the satellite DNA sequences to Japanese quail chromosomes using the 192 bp fragment as a biotinylated probe (Figure 3). The satellite DNA was distributed in the centromeric region of a large majority of microchromosomes and in the telomeric regions of the short arms of telocentric chromosomes 4 and W. We observed 10 metaphase spreads hybridized with the satellite DNA in a good condition. About 50 of the 66 microchromosomes have positive signals, however, exact counts were not always possible because of their small sizes.

Discussion

We molecularly cloned a novel satellite DNA sequence of Japanese quail directly from the satellite DNA bands, which were revealed by gel electrophoresis of BglII-digested genome DNA. The sequences consisted of 41 bp tandemly repeated elements, of which GC content was relatively high (~61%), and they were predominantly concentrated in the centromeric heterochromatin of microchromosomes (Figure 3A). A sequence homology search (using BLAST in GenBank and FASTA search in DDBJ) did not show any related DNA sequences entered in the GenBank and DDBJ.

Analytical ultracentrifugation of Japanese quail DNA by Comings and Mattoccia (1970) showed a satellite band with a buoyant density of 1.715 g/cm³ and its GC content was estimated at 55% by both buoyant density and DNA TM. Brown and
Jones (1972) reported that the 1.715 g/cm³ satellite DNA fraction was quite rich in the microchromosome of Japanese quail, but was not present in the chicken. The characteristics of the 41 bp tandemly repeated sequence shown in this study were in good agreement with those of 1.715 g/cm³ satellite DNA of Japanese quail (Brown and Jones 1972). It was reported that most of the microchromosomal heterochromatin of Japanese quail was GC rich because it was readily stained with GC-specific fluorescence dye, chromomycin A₂, and R-band positive for acridine-orange staining (Comings and Wyandt 1976; Mayr et al. 1989). Microchromosomes of Japanese quail were not well stained with AT-specific dye Hoechst 33258 (Figure 3B). These observations were consistent with the characteristics of the 41 bp tandemly repeated sequence cloned in this study, that is, relatively high GC contents (~61%) and predominant distribution in centromeric heterochromatin of microchromosomes. These results suggest that this satellite DNA is a major component of microchromosomal heterochromatin of Japanese quail.

In the Galliformes species, 41–42 bp tandem repeat sequences that were enriched in microchromosomes were isolated from chicken (CNM repeat) and turkey (TM repeat) (Matzke et al. 1990, 1992). The microchromosomal distribution patterns of these satellite DNAs are quite coincident with that of Japanese quail-specific satellite DNA isolated in this study. The CNM and TM repeats are AT rich (56–63%), share an alternating A₃T₃ and T₃A₃ direct repeat motif sequences, and form hairpin loop structures (Matzke et al. 1990, 1992), while the Japanese quail satellite DNA is GC rich and does not form such hairpin loop structures (Figure 1C). Because of such a large difference of DNA sequences, it is not clear if the 41 bp microchromosomal repetitive sequences of chicken and Japanese quail evolved from a common ancestor. It is known that microchromosomes are late replicating in Japanese quail, but they are early replicating in chicken (Bianchi and Molia 1967; Comings and Mattoccia 1970; McQueen et al. 1998; Schmid 1962). This difference in DNA replication patterns of microchromosomes is assumed to be caused by the divergence of the sequences of the satellite DNAs in microchromosomes. A detailed study of microchromosomal satellite DNAs would help elucidate genetic divergence of microchromosomes in the process of speciation in Galliformes.

This satellite DNA was localized primarily to microchromosomes, but was also located on the telomeric regions of short arms of chromosomes 4 and W (Figure 3). The positive hybridization signals of the satellite sequence on both microchromosomes and the W chromosome in Japanese quail provides support for the hypothesis that these repetitive sequences have a common origin. Matzke et al. (1992) report that in chicken and turkey, microchromosomal satellite DNA and a W-specific repeat share an ancestral 21 bp element. Stock and Bunch (1982) compared G- and C-banding patterns of microchromosomes between Japanese quail and chicken, and indicated that the chromosome 4 of Japanese quail had an additional small heterochromatic short arm on which the hybridization signals of the “microchromosomal” satellite DNA were located (Figure 3). This result suggests that the short arm of chromosome 4 of Japanese quail was formed by a translocation of microchromosomal heterochromatin or by a tandem fusion of a microchromosome.

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