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Inferring gene flow in coral reef fishes from different molecular markers: which loci to trust?

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ABSTRACT

Contrasting results are usually reported in the literature regarding the factors influencing observed structuring of genetic variability. The goals of this study were, for five coral reef fishes in French Polynesia, (1) to infer the theoretical variance of single locus F_{ST} estimates expected under neutrality in order to exclude outlier loci before inferring gene flow and (2) to test thereafter whether species laying pelagic eggs effectively disperse more than species laying benthic eggs in this system. For this purpose, a total of 952 individuals from five species belonging to two families (Chaetodontidae and Pomacentridae) were screened among populations sampled within a 60–600km spatial range for intron length polymorphism at 11 loci in order to illuminate contrasting results previously published on allozymes and mitochondrial DNA (mtDNA) control region polymorphisms. Statistically speaking, among the five species, four loci (three allozymes and one intron) were identified as outliers and discarded before interpretation of genetic differentiation in terms of effective dispersal. Biologically speaking, our results suggest that the observed genetic structure is not significantly related to the reproductive strategy of coral reef fish in the island system we analysed and that observed random genetic differentiation accommodates Wright's island model in all five species surveyed. Overall, our study emphasizes how cautious one has to be when trying to interpret present-day genetic structure in terms of gene flow while using a limited number of loci and/or different sets of loci.

KEYWORDS

coral reef fishes; allozymes; introns; mtDNA; gene flow; neutrality

INTRODUCTION

Studying spatial partitioning of genetic variation is viewed as a robust method for inferring gene flow among discrete populations, conditional on taking into account all processes shaping the present genetic structure (Hewitt, 2000). For coral reef fishes, contrasting results have been found relative to the relationship between present genetic structure and larval duration (Waples, 1987; Doherty et al., 1995; Shulman and Bermingham, 1995; Rocha et al., 2002; Bay et al., 2006), reproductive strategy (Shulman and Bermingham, 1995; Planes et al., 1997), species swimming abilities (Waples, 1987; Riginos and Victor, 2001; Purcell et al., 2006) or geographic distances (Planes et al., 1993, 1996; Planes and Fauvelot, 2002; Purcell et al., 2006). Fauvelot and Planes (2002) have shown, using allozymes, that the observed significant structuring of genetic variation among populations of eight coral reef fishes in a fragmented island system was not correlated with geographic distances at the studied spatial scale (ca 1500 km) nor associated with species reproductive strategy and larval duration. On average, only a third of the polymorphic loci contributed to the overall genetic differentiation. Conversely, the analysis of the genetic variation at the mitochondrial DNA (mtDNA) control region sequences in a subset of individuals revealed genetically homogeneous populations in all seven species under study (Fauvelot et al., 2003).

Such puzzling discordant patterns of genetic variation obtained at different loci are increasingly described (Karl and Avise, 1992; Pogson et al., 1995; Lemaire et al., 2000; De Innocentiis et al., 2001; Dufresne et al., 2002; Arnaud-Haond et al., 2003; Gomez-Uchida et al., 2003; Nielsen et al., 2006) and lead to question the interpretation made of genetic differentiation (F_{ST}) estimates in terms of effective dispersal. A key issue is that, under neutrality, all loci should theoretically be affected similarly by drift and migration (Lewontin and Krakauer, 1973; Slatkin, 1987), so that F_{ST} estimates should be homogeneous among loci, and multilocus F_{ST} estimates may be used to infer gene flow among populations. Yet, erroneous gene flow estimates may result from the interpretation of allelic distribution over multiple loci for two main reasons. One lies in the underlying high variance of the migration/drift process itself and its sensitivity to departure from equilibrium (Whitlock and McCauley, 1999). The other resides in selection or other non-neutral mechanisms (acting directly or through hitch-hiking) differentially affecting loci (Cavalli-Sforza, 1966; Slatkin, 1987). Indeed, disruptive selection will result in higher F_{ST} estimates than expected under neutrality, inflating the overall genetic differentiation. On the other hand, loci experiencing balancing selection will have allele frequencies more similar than expected under neutrality, reducing the F_{ST} estimates and further giving the false view of high gene flow.

In order to properly infer gene flow among populations, it appears therefore essential to (1) estimate genetic differentiation based on reasonable numbers of individuals (50–100 individuals; Ruzzante, 1998), loci (15–20 loci; Nielsen et al., 2006) and alleles (either few loci with many alleles or many loci with a few alleles; Kalinowski, 2002) and (2) discard loci behaving non-neutrally before the estimation of genetic differentiation. Several neutrality tests are available in the current literature that may allow detection of selected loci at ecological timescales (reviews in Guinand et al., 2004; Beaumont, 2005). Among these tests, those derived from the Lewontin–Krakauer method (that is, based on multilocus comparisons and the identification of F_{ST} outliers) appear robust when allele distributions are available at multiple loci (Beaumont and Nichols, 1996; Beaumont and Balding, 2004; Beaumont, 2005).

The goal of the present study is, for five species of coral reef fish, to infer gene flow among insular populations sampled within a 60–600km spatial range and to test for the influence of reproductive strategies. Considering the problem of neutrality among loci, the

theoretical neutral variance of single locus F_{ST} estimates in our insular system was first investigated in order to identify (and exclude from calculation) outlier loci before computing gene flow. For this purpose, the number of loci was increased to improve the power of the test. We reanalysed all previously assayed individuals from five species with a new set of loci with potentially higher mutation rates and gene diversities than allozymes, that is, intron length polymorphisms. Unlike exons, the nucleotide sequences of introns are little constrained so that introns accumulate mutations rapidly. Therefore, introns often display a high genetic variability (Palumbi, 1995), which make introns being targeted as markers of population variation and subdivision (Lessa, 1992; Borsa, 2003; Hassan et al., 2003; Friesen et al., 2005; Rohfritsch and Borsa, 2005; Berrebi et al., 2006). Exon-primed, intron-crossing polymerase chain reaction (EPIC-PCR) amplification is an appreciable strategy for finding DNA polymorphism in several species of different families (Hassan et al., 2002) and an alternative to the development of microsatellite loci. Length polymorphism at 11 intron loci was assessed in five coral reef fish species and the corresponding new data set was further added to the previous allozymes data set (Fauvelot and Planes, 2002) to conduct global analyses. The comparison of the distribution of genetic variation obtained from different markers using the same set of individuals from related species collected in the same area also provide a solid framework to test among mutation, migration and genetic drift as the main forces driving the distribution of genetic polymorphisms.

MATERIALS AND METHODS

Sampling

Sampling was carried out as described in Fauvelot and Planes (2002). Briefly, five different islands were sampled across French Polynesia based on a standard sampling effort of 50 individuals per island per species. Because the eight species selected for the original study were not all present in the five islands, and in order to compare effective dispersal among species at similar scales, we restricted further analysis to five species inhabiting four common islands: Rangiroa, Takapoto, Tetiaroa and Moorea (Figure 1). Geographic distances between islands ranged from 60km (between Moorea and Tetiaroa) to 566km (between Moorea and Takapoto). *Chaetodon citrinellus* is a butterflyfish strictly inhabiting lagoons while *C. quadrimaculatus* and *Forcipiger flavissimus* are both outer reef butterflyfishes. *Dascyllus aruanus* and *Pomacentrus pavo* are two damselfishes strictly inhabiting lagoons. Fishes were collected, tissue stored and DNA extracted as described in Fauvelot et al. (2003). A total of 952 individuals from five species (Table 1) were used for further genetic screening.

Genetic analysis – EPIC-PCR

Eleven introns loci were tested for length polymorphism for each species: intron 7 of creatine kinase; intron 2 of glyceraldehyde-3-phosphate dehydrogenase; introns 2 and 5 of growth hormone; introns 1, 2, 4 and 5 of aldolase B; introns 1, 2 and 3 of gonadotropin-releasing hormone (Hassan et al., 2002). PCR amplification and electrophoresis follow protocols described in Hassan et al. (2002). Briefly, amplified PCRs (with forward primer labelled radioactively with ^{32}P) were loaded on a 6% polyacrylamide gel in TBE buffer. The gels were run for 3 h at 50W. After drying, the gels were exposed against films of Biomax autoradiograph. Individual genotypes were directly scored from the autoradiograph films with alleles being identified according to their size relative to the most common allele, as for allozymes. To ensure scoring accuracy and consistency across gels and populations, a locus-specific size standard from a pool of individuals with known genotypes was run at both sides

of each gel. With this method, we were able to segregate 1 bp length difference bands. Individuals for which no PCR products were visualized on gel were further re-amplified in a new PCR using a 2°C lower annealing temperature. Results of the amplification successes across species are presented in Hassan et al. (2002) and detailed PCR conditions for each species and locus are available from the authors.

Data analysis

The allozymes data set was reanalysed for genetic divergences in the overall populations following the removal of populations from the data set (see sampling). Allele frequencies, number of alleles per locus (N_A), observed heterozygosity (H_O) and unbiased gene diversity (H_E ; Nei, 1987) were calculated within each population and over all populations for both allozymes and introns. Linkage disequilibrium between loci was tested using the procedure of Black and Krafur (1985). Deviation from Hardy–Weinberg expectations (HWE) at introns loci was measured using Weir and Cockerham (1984) inbreeding coefficient f (equivalent to Wright's F_{IS}), and locus conformance to HWE was assessed by permuting alleles within populations (1000 permutations). The presence of null alleles was tested using the program MICRO-CHECKER (Van Oosterhout et al., 2004). Genetic divergences were estimated using the Weir and Cockerham (1984) estimator. For each species, overall single locus F_{ST} values were estimated by jackknifing over populations and overall global F_{ST} estimates were obtained by jackknifing over loci. Unless specified, all computations were conducted using GENETIX (Belkhir et al., 2002).

The theoretical distribution of single locus F_{ST} estimates expected under neutrality was computed through simulations and outlier loci identified using Beaumont and Nichols' (1996) test. The principle of this test is to estimate, conditional on gene diversity, the range of F_{ST} values expected under the combined effect of migration and genetic drift in a model approaching the studied system, and to compare the observed F_{ST} at this given locus to the simulated empirical distribution. The models for computer simulations were chosen as to fit at maximum the conditions of the studied system. Indeed, we chose the infinite allele model of evolution (Kimura and Crow, 1964), and the infinite island model of Wright (1943) as this is the best-suited available model for fragmented insular environments such as Pacific reefs. It should be noted that, for heterozygosities lower than 0.8 (which is our case), stepwise and infinite-allele models lead to similar simulated distributions of F_{ST} (Beaumont and Nichols, 1996), so that the choice of the mutation model has little impact on the simulated empirical distribution.

Coalescent simulations were performed to generate data sets with a distribution of F_{ST} close to the empirical distribution. Simulations were conducted assuming four sampled populations, with a metapopulation size of 100 populations (the approximate number of islands in French Polynesia), sample sizes of 50 individuals and an expected F_{ST} equal to the median estimate of F_{ST} calculated for all loci. Under the infinite allele mutation model, 20 000 H_E and F_{ST} paired values were generated and the P -values for each locus were then calculated using the procedure described in Beaumont and Nichols (1996). Loci with unusually low or high F_{ST} estimates (that is, out of the 95% distribution of simulated F_{ST}), conditional on heterozygosity, were regarded as outlier loci. We used the currently distributed version of the test, namely F_{dist2} (<http://www.rubic.reading.ac.uk/~mab/software/fdist2.zip>), described in Beaumont and Balding (2004). Because the test conducts simulations based on initial expected F_{ST} value set up by the user, this value was first fixed equal to the global F_{ST} (estimated over all loci). However, because this median value may be biased owing to the presence of an outlier locus,

we systematically conducted again the simulations with a new global F_{ST} estimated omitting the outlier locus (or loci), and so on until no more loci were identified as outliers.

Overall populations and pairwise F_{ST} were estimated for each species over all nuclear loci (allozymes and introns) once removing outliers detected by the Beaumont and Nichols' (1996) test. Significance of pairwise genetic divergence was tested using a permutation procedure (1000 permutations) in GENETIX (Belkhir et al., 2002). Mantel tests were performed to test for a correlation between geographical distance and multilocus F_{ST} (approach of Hutchison and Templeton, 1999). For each species, Mantel coefficient Z was computed and tested for significance through a permutation procedure with 100 permutations, under the null hypothesis that both matrices are not linearly related. To evaluate the level of correlation between genetic patterns exhibited by the different species, matrices of pairwise F_{ST} were compared with a Mantel test: Pearson correlation coefficient r was computed for each pair of species and its significance tested using a permutation procedure with 100 permutations. All tests were performed in GENETIX using the Mantel option.

RESULTS

Out of the 11 intron primer pairs tested, five loci (*AldoB1*, *AldoB5*, *CK7*, *GnRH3-3* and *Gpd2*) could not be further used for the five species because they lead to multibanded (several loci amplified), monomorphic or not clearly interpretable patterns (Table 2). The remaining six loci (*AldoB2*, *AldoB4*, *GnRH3-1*, *GnRH3-2*, *GH2* and *GH2*) provided single locus amplifications with clearly interpretable length polymorphism, and finally, 2–3 polymorphic intron per species could be analysed (Table 2). Over all populations, the number of alleles per locus ranged from 2 to 12 (for *GH2* over all *F. flavissimus* sampled populations). Gene diversity among populations in each species was more or less even and variations in gene diversity were locus specific, such as that observed at enzymatic loci (Fauvelot and Planes, 2002), with no congruent patterns among species. Over all four populations, gene diversity at intron loci reached 0.726 for *F. flavissimus* at *GH2*, and 0.501 at enzymatic loci for *F. flavissimus* at *Est1* (Table 3).

After standard Bonferroni correction of the 5% significance level, a single locus significantly deviated from HWE; *GnRH3-1* in *C. citrinellus* populations. The cause of such departures is likely to be the presence of null alleles because the deviations resulted from significant heterozygote deficiencies, observed in all populations in this locus only. The occurrence of null alleles was further confirmed using MICRO-CHECKER and the average null allele frequency at *GnRH3-1* locus across populations was 0.165 (estimator of Brookfield, 1996). However, this locus was maintained for further analysis, and genotypes were not adjusted according to nulls to allow for multilocus analysis.

Significant ($P < 0.01$) linkage disequilibria were found between introns originating from the same gene (that is, *GnRH3-1* and *GnRH3-2* for *C. citrinellus* and *C. quadrimaculatus*; between *AldoB2* and *AldoB4* for *D. aruanus*). For *C. citrinellus*, the association was significant between the two most common alleles at each locus, and between rare alleles at *GnRH3-1* with two of the four alleles at *GnRH3-2*. For *C. quadrimaculatus* and *D. aruanus*, the linkage involved rare alleles from one locus significantly associated with common alleles from the other locus.

However, all loci were maintained for further analysis.

For each species, over all four populations, a wide range of single locus F_{ST} was observed among nuclear loci (allozymes and introns), from negative values up to 0.198 at *ADA-1* for *D. aruanus*. Multilocus F_{ST} estimates ranged from 0.008 for *C. citrinellus* to 0.026 for *D. aruanus*

(Table 3). Discrepancies among classes of markers were observed because only at enzymatic loci, global F_{ST} estimates ranged from 0.012 for *C. citrinellus* to 0.031 for *F. flavissimus* and *D. aruanus*, while when looking at only introns, global F_{ST} estimates were zero for all species except one, *F. flavissimus* ($F_{ST} = 0.008$, $P > 0.05$). However, these discrepancies were not marker related but locus related because single locus F_{ST} estimates at introns were similar to those of most of allozymes within species (Table 3).

Beaumont and Nichols' (1996) test revealed that three loci demonstrated significantly higher F_{ST} values than expected under the null hypothesis of neutrality: *ADA-1* in *D. aruanus* ($P = 0.991$), and *ADA* and *GDA* in *F. flavissimus* ($P > 0.999$ and $P = 0.953$, respectively) (Figure 2). One additional locus, *AldoB4* in *P. pavo*, showed significantly lower F_{ST} values ($P = 0.037$) than expected under neutrality. Recalculating the global F_{ST} for each species without the outliers loci did not induce further rejection of neutrality for any other locus.

Excluding outlier loci detected by Beaumont and Nichols' (1996) test, global F_{ST} were significant for all five species and ranged from 0.008 ($P = 0.01$) for *C. citrinellus* to 0.019 ($P = 0.02$) for *D. aruanus* (Table 4). Pairwise F_{ST} estimated from all nuclear loci excluding outliers ranged from 0 to 0.045 (between Rangiroa and Moorea for *P. pavo*; Table 4). None of the five species expressed significant correlation between genetic divergences and geographic distances between populations (all $P > 0.05$). The comparison of pairwise F_{ST} matrices among the five species revealed that two species showed a significant correlation of their patterns of genetic structuring throughout the four sampled islands: *C. quadrimaculatus* and *D. aruanus* ($r = 0.843$, $P = 0.03$; see Table 4).

DISCUSSION

FIGURE 1

Location of sampling sites of the five fish species in French Polynesia (South Pacific Ocean). Details of sites are reported in Fauvelot and Planes (2002) and sampling effort is indicated in Table 1.

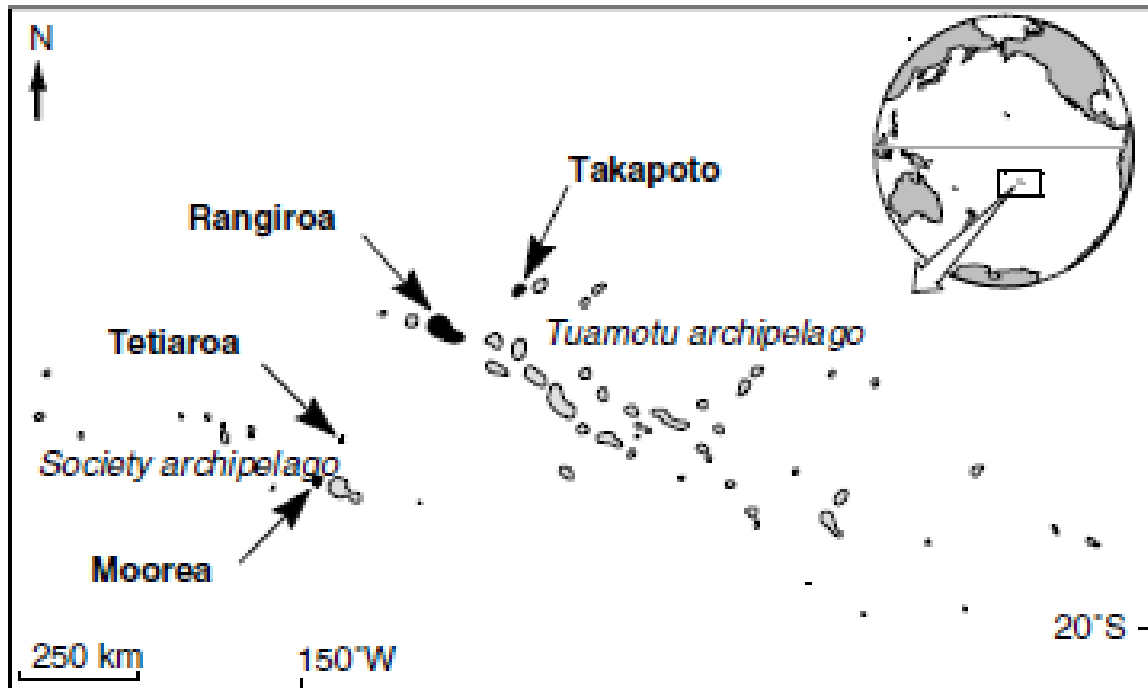


FIGURE 2

Summary of the results of the Beaumont and Nichols' (1996) neutrality test. For each locus, estimate of F_{ST} is plotted against its corresponding transformed P -value (expressed as $\text{logit}(2|P-0.5|)$) as in Beaumont and Balding, 2004), corresponding to the rejection of the null hypothesis of neutrality. The vertical dotted line bar shows the critical transformed P -values used for identifying outlier loci at the 5% confidence level. Each outlier locus is denoted as follows: the two first letters refer to the species (*Da*, *Dascyllus aruanus*; *Ff*, *Forcipiger flavissimus*; *Pp*, *Pomacentrus pavo*), followed by the locus name.

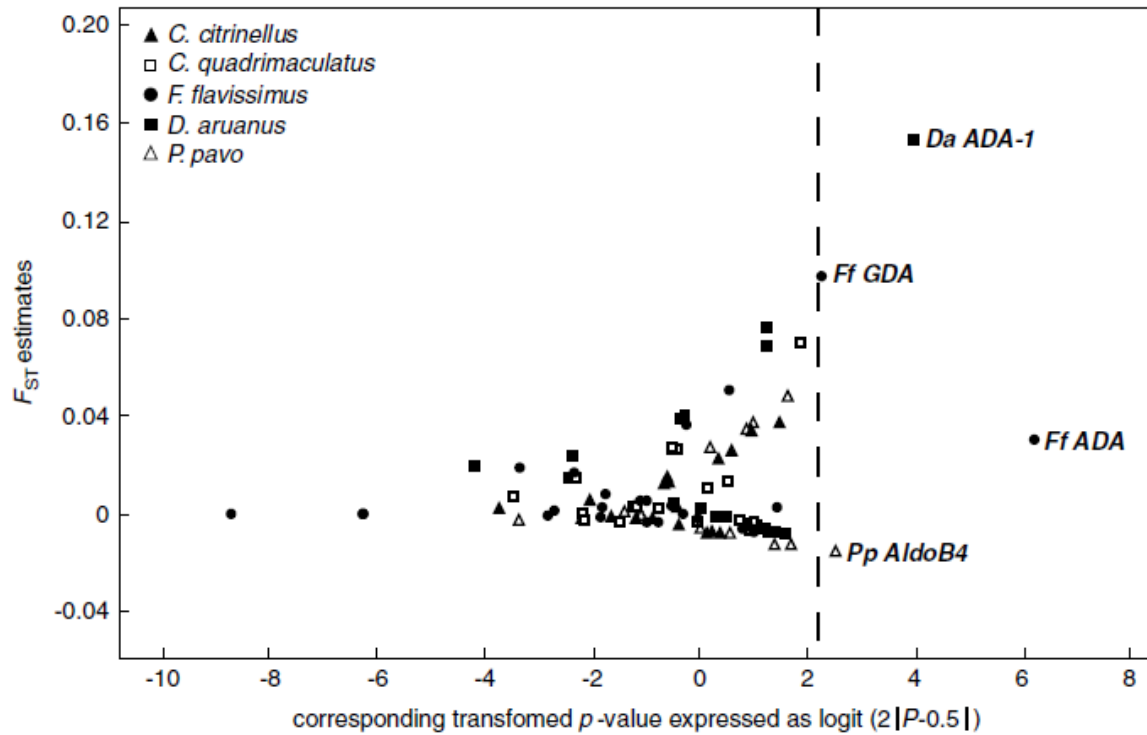


TABLE 1

Number of individuals scored at enzymatic and introns loci, indicated per species and per site

<i>Species</i>	<i>Rangiroa</i>	<i>Takapoto</i>	<i>Tetiaroa</i>	<i>Moorea</i>	<i>Total</i>
<i>Chaetodon citrinellus</i>	50	50	50	50	200
<i>Chaetodon quadrimaculatus</i>	50	48	49	49	196
<i>Forcipiger flavissimus</i>	50	50	49	39	188
<i>Dascyllus aruanus</i>	46	48	50	49	193
<i>Pomacentrus pavo</i>	27	50	48	50	175

TABLE 2

Amplifications results of the 11 tested

	Chaetodon citrinellus				Chaetodon quadrimaculatus				Forcipiger flavissimus				Dascyllus aruanus				Pomacentrus pavo			
	Ra	Ta	Te	Mo	Ra	Ta	Te	Mo	Ra	Ta	Te	Mo	Ra	Ta	Te	Mo	Ra	Ta	Te	Mo
<i>AldoB1</i> H _E	M	M	M	M	M	M	M	M	M	M	M	M	Poly	Poly	Poly	Poly	Poly	Poly	Poly	Poly
<i>AldoB2</i> H _E H _O N _A	0.115 0.120 4	0.078 0.080 4	0.099 0.102 4	0.098 0.061 4	M	M	M	M	M	M	M	M	0.145 0.152 3	0.082 0.083 3	0.217 0.160 3	0.136 0.143 3	M	M	M	M
<i>AldoB4</i> H _E H _O N _A	M	M	M	M	M	M	M	M	M	M	M	M	0.472 0.421 2	0.428 0.478 2	0.421 0.409 2	0.467 0.511 2	0.492 0.444 2	0.494 0.567 2	0.494 0.483 2	0.481 0.460 2
<i>AldoB5</i> H _E CK7 H _E	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	M Multi	nt nt	nt nt	nt nt	nt nt	nt nt	nt nt	nt nt	nt nt
<i>GnRH3-1</i> H _E H _O N _A	0.702 0.480** 8	0.628 0.240** 5	0.657 0.388** 7	0.693 0.449** 8	0.589 0.553 5	0.636 0.667 5	0.627 0.636 5	0.552 0.556 6	M	M	M	M	M	M	M	M	M	M	M	M
<i>GnRH3-2</i> H _E H _O N _A	0.518 0.400* 4	0.490 0.460 4	0.565 0.510 4	0.524 0.347** 4	0.549 0.583 3	0.473 0.512 3	0.539 0.400* 3	0.556 0.575 4	0.443 0.396* 7	0.219 0.234 6	0.502 0.500 7	0.456 0.400 5	M	M	M	M	M	M	M	M
<i>GnRH3-3</i> H _E	M	M	M	M	M	M	M	M	M	M	M	M	Poly	Poly	Poly	Poly	M	M	M	M
<i>GH2</i> H _E H _O N _A	M	M	M	M	M	M	M	M	0.774 0.745 11	0.726 0.714 11	0.746 0.838 8	0.658 0.615 8	Multi	Multi	Multi	Multi	Multi	Multi	Multi	Multi
<i>GH5</i> H _E H _O N _A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	0.373 0.333 2	0.364 0.400 2	0.300 0.353 2	0.311 0.260 2
<i>Gpd2</i> H _E	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M

Abbreviations:

HE, unbiased gene diversity (Nei, 1987); HO, observed heterozygosity; M, monomorphic (no length polymorphism); Mo, Moorea; Multi, multiband pattern (more than one locus amplified); NA, number of alleles; nt, not tested; Poly, length polymorphism but hardly interpretable; Ra, Rangiroa; Ta, Takapoto; Te, Tetiaroa.

Intron names are given as in Hassan et al. (2002).

Significant deviation from Hardy–Weinberg proportions is denoted by * for $P < 0.05$ and ** for $P < 0.01$.

TABLE 3

Gene diversity (HE) over all four sampled populations and FST estimates for each scored locus (allozymes and introns) per species

	C. citrinellus		C. quadrimaculatus		F. flavissimus		D. aruanus		P. pavo	
	H _E	F _{ST}	H _E	F _{ST}	H _E	F _{ST}	H _E	F _{ST}	H _E	F _{ST}
<i>Allozymes</i>										
AAT-1	0.273	-0.005	0	—	0.091	0.000	0	—	0	—
AAT-2	0.216	-0.007	0.020	0.020	0.108	0.005	0.196	-0.009	0	—
ADA-1	0.511	0.012	0.005	0.000	0.019	0.030	0.191	0.198	0.005	-0.002
ADA-2							0.371	0.066		
ADH					0.005	-0.001				
CK	0	—	0	—	0.005	-0.001	0	—	0	—
Est1			0.326	0.025	0.501	0.012	0.358	0.019	0.254	0.047
Est2	0.481	0.014	0.050	-0.004	0.224	0.001			0.208	-0.004
Est3			0.473	0.070						
EstD					0.036	0.006				
GDA	0.345	0.036	0.375	0.025	0.444	0.088				
GDH					0.268	-0.007				
GPD-1	0.020	-0.001	0.015	0.002	0.015	0.001	0.214	0.030		
GPD-2					0.010	0.004	0.070	0.000	0.422	-0.009
GPI-1	0.078	0.002	0.134	0.002	0.010	-0.007	0.475	-0.004	0	—
GPI-2	0.020	-0.001	0.015	0.002	0.043	-0.001	0.031	-0.008	0	—
IDH-1	0.374	-0.001	0.005	0.000	0.012	-0.002	0.127	-0.008	0	—
IDH-2	0	—	0.020	-0.004	0.145	0.038	0.116	0.050	0.100	-0.011
LDH	0.121	0.019	0	—	0.017	0.001			0	—
MDH-1	0	—	0.005	0.000					0	—
MDH-2	0	—	0.010	0.010	0	—	0.103	0.000		
MEP-1	0.068	0.001	0.074	-0.009	0.015	-0.002	0.270	0.014	0.010	-0.004
MEP-2	0	—	0.264	0.026	0	—	0	—	0.381	0.042
MPI	0	—	0	—	0.036	-0.006			0.010	-0.004
PepB-1	0	—	0	—			0.390	0.019	0.005	-0.001
PepB-2	0.181	0.032	0.035	0.008	0.011	-0.005			0.005	-0.002
PepD-1	0.401	0.023	0.005	0.000	0.105	-0.003	0.294	0.077	0.014	0.000
PepD-2									0.322	0.028
PGAM	0	—	0.005	0.000					0.406	0.026
PGDH	0.098	0.027	0.390	0.016	0.021	0.000	0	—		
PGM	0.261	-0.008	0.005	0.000	0.405	0.048	0.108	-0.006	0.009	0.009
SDH							0.178	-0.002		
SOD-1	0	—	0.005	0.000					0	—
SOD-2			0.020	-0.003	0.005	-0.001				
XO									0.181	-0.009
<i>Introns</i>										
AldoB2	0.097	-0.009	0	—	0	—	0.447	-0.005	0	—
AldoB4	0	—	0	—	0	—	0.145	0.004	0.490	-0.014
GnRH3-1	0.670	-0.001	0.601	-0.004	0	—	0	—	0	—
GnRH3-2	0.524	-0.004	0.529	-0.004	0.405	0.017	0	—	0	—
GH2	0	—	0	—	0.726	0.002				
GH5	0	—	0	—	0	—	0	—	0.337	-0.011
<i>Overall polymorphic loci</i>										
Allozymes	0.230	0.012	0.103	0.030	0.106	0.031	0.218	0.031	0.156	0.018
Introns	0.430	-0.003	0.565	-0.004	0.566	0.008	0.296	-0.003	0.413	-0.014
Overall	0.263	0.008	0.141	0.018	0.142	0.023	0.227	0.026	0.186	0.010

TABLE 4

Pairwise genetic differentiation (FST) estimated among islands excluding outlier loci

	<i>km</i>	<i>C. citrinellus</i>	<i>C. quadrimaculatus</i>	<i>F. flavissimus</i>	<i>D. aruanus</i>	<i>P. pavo</i>
Ta/Mo	566	0.011	0.018	0.030	0.016	0.024
Ta/Te	520	0.011	0.005	0.018	0.011	0.000
Ra/Mo	365	0.000	0.019	0.022	0.014	0.045
Ra/Te	310	0.007	0.016	0.004	0.026	0.022
Ra/Ta	226	0.001	0.012	0.002	0.011	0.000
Te/Mo	60	0.017	0.036	0.005	0.035	0.006
Overall		0.008	0.018	0.013	0.019	0.014

Abbreviations: Mo, Moorea; Ra, Rangiroa; Ta, Takapoto; Te, Tetiaroa.

Significant values after sequential Bonferroni correction are indicated in bold.

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