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Genetic variation in the lesser flying squid *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) in east Atlantic and Mediterranean waters

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ABSTRACT: Samples of *Todaropsis eblanae* from throughout the species range in east Atlantic and Mediterranean waters were screened for genetic variability at 1 minisatellite and 4 microsatellite loci. Extremely high levels of variability were observed within samples at all loci, with the mean observed heterozygosity per locus ranging from 0.82 to 0.91. Tests of allele frequency heterogeneity and measures of F_{ST} (Wright's fixation index) suggest that significant genetic differentiation occurs between samples taken in African waters and those taken in European waters (overall $F_{ST} = 0.014$, $p < 0.001$). Furthermore, significant differentiation was evident between the samples taken from South African waters and those taken from off Mauritania (between sample $F_{ST} = 0.012$, $p < 0.001$). More subtle structuring was suggested within the European samples, largely attributable to differences between the Mediterranean sample and Atlantic samples (within Europe $F_{ST} = 0.002$, $p < 0.03$). However, the high levels of heterozygosity associated with the examined loci means that biological implications of marginally significant statistical results must be considered. One consistent aspect of the observed variation is that the Mauritanian sample is more genetically differentiated from European samples than from the more geographically isolated South African sample. Homoplasy and historical events such as climatic cycles and post-glacial recolonisation are considered as possible explanations for the observed patterns of variation. In terms of stock structure, the findings suggest the presence of at least 3 genetically isolated populations in the east Atlantic, which has important implications for management and sustainability of this resource.

KEY WORDS: *Todaropsis eblanae* · Squid · Microsatellites · Minisatellites · Population genetics · Fisheries · Stocks

INTRODUCTION

In recent years cephalopod fisheries have come to form a substantial component of global landings as reported by the FAO (www.fao.org), and have been considered by some as being among the few fisheries which still hold potential for expansion (Caddy & Rodhouse 1998, Lordan 2001). The lesser flying squid *Todaropsis eblanae*, Ball 1841 belongs to the family Ommastrephidae (or short-finned squid), which in terms of fisheries, are considered among the most important cephalopods (Nesis 1987, Nigmatullin 2002) and account for up to 70% of world catch. Recent ICES (International Council for the Exploration of the Seas) (Anonymous 2003), and FAO landing statistics indicate that overall NE Atlantic catch of short-finned squid (including *T. eblanae*, *Illex coindetii* and *Todarodes sagittatus*) has increased between 1996 and 2000, suggesting that cephalopods are gaining importance as a fishery resource. Serious declines

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in abundance of many finfish and large reductions of commercial quotas have initiated the search for other species for exploitation. While the potential for development of targeted or semi-targeted fisheries for this species is uncertain (Lordan 2001), traditional finfish fisheries could be expanded to exploit this alternative resource.

Todaropsis eblanae is widely distributed throughout east Atlantic waters occurring from the North Sea to South African waters and in the western Mediterranean (Roper et al. 1984). The species has also been reported from the Indian Ocean, Australian waters (Roper et al. 1984), the west Atlantic (Lipinski et al. 1992) and the south China Sea (C. C. Lu, National Chung Hsing University, Taiwan, pers. comm.). Studies in recent years have investigated aspects of ecology, reproductive biology, age and growth of *T. eblanae* in Atlantic and Mediterranean waters (Lipinski 1992, Lipinski et al. 1993, Gonzalez et al. 1994, Hastie et al. 1994, Rasero 1994, 1996, Rasero et al. 1996, Lordan et al. 1998, Arkhipkin & Laptikhovsky 2000, Hernandez-Garcia 2002, Robin et al. 2002). While migratory behaviour in this species remains poorly understood, observations on morphometrics by Lordan (2001) suggest that *T. eblanae* may be more similar to neritic loliginid squid species which tend to be less migratory than sympatric ommastrephid species (e.g. *Illex coindetii*, *Todarodes sagitattus*). To date, no information has been published regarding the genetic population structure and levels of differentiation between stocks of *T. eblanae*. Without such data, effective management strategies for this emerging resource will be severely limited.

Molecular markers are increasingly being employed to acquire data on population genetics and stock structure of marine species (e.g. Magoulas et al. 1996, Chikhi et al. 1998, O'Connell et al. 1998, Adcock et al. 1999, Shaw et al. 1999, Murphy et al. 2002, Perez-Losada et al. 2002, Shaw 2002) and microsatellite DNA loci are generally the markers of choice, due to their high levels of variability. Earlier genetic studies of cephalopods using allozyme markers had failed to provide a reliable indication of stock structure due to the low levels of genetic variability observed at these loci (Garthwaite et al. 1989, Carvalho et al. 1992, Brierley et al. 1995). However, more recent studies (Adcock et al. 1999, Shaw et al. 1999, Murphy et al. 2002, Perez-Losada et al. 2002, Shaw 2002) indicate that better resolution of population structure is possible using microsatellite DNA markers.

Microsatellite markers have been designed specifically for *Todaropsis eblanae* (Dillane et al. 2000), and in the present study these are used to assess population structure of this species throughout its range in east Atlantic and Mediterranean waters.

MATERIALS AND METHODS

Samples. Samples for microsatellite analysis were selected from collections of ethanol-preserved material provided by different partners in a European research project (FAIR-CT96-1520). Sample sizes ranged from 31 to 70 individuals, and were collected from a combination of trawl and market surveys. All samples were collected during 1998, with the exception of the west Mediterranean and South African samples which were obtained in 1999. Fig. 1 illustrates collection areas and sample sizes.

Microsatellite amplification and detection. Prior to microsatellite analysis, genomic DNA was extracted from ethanol-preserved armtip tissue using a CTAB method as described by Winnepenninckx et al. (1993). All samples were screened for variability at 4 microsatellite loci (Tebl20, Tebl32, Tebl25, Tebl812) and 1 minisatellite locus (Teblmini) (Dillane et al. 2000). PCR amplifications were carried out as described by Dillane et al. (2000), where one of the primers was end-labelled with IRD800 (MWG BIOTECH™). PCR products were resolved using a LiCOR™ automated DNA sequencer on 6% denaturing polyacrylamide gels.

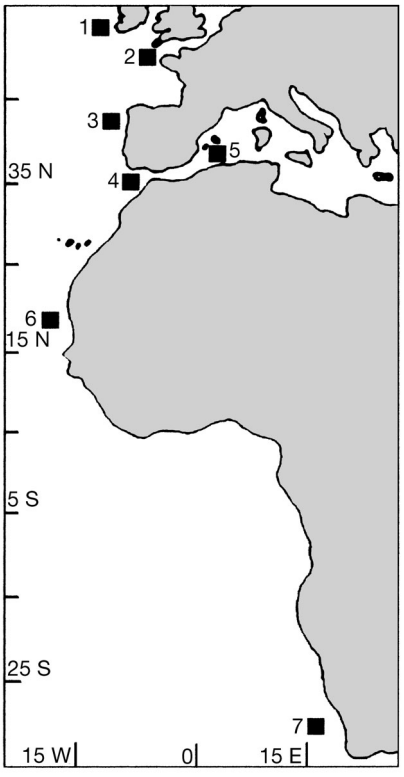

Fig. 1. *Todaropsis eblanae*. Study area and sampling locations. 1: west of Ireland (Feb 98, n = 50), 2: English Channel (Oct 98, n = 31), 3: Galician coast (Sept 98, n = 60), 4: Algarve (June 98, n = 45), 5: west Mediterranean (June 99, n = 60), 6: Mauritania (Oct 98, n = 60), 7: South Africa (April 99, n = 70)

Allele sizes were determined using a combination of a molecular weight marker (LiCOR™) and standard alleles to ensure accurate and consistent scoring of genotypes. Gene Imagir (Scanalytics™) software was used to assign allele sizes. In the case of locus Teblmini, a very large number of alleles were detected (190+) ranging in size from 200 to 1500 bp, creating difficulties in determining allele size with a high degree of certainty. Therefore, alleles were 'binned' into 36 size classes by assessing allele size distributions in histograms and establishing where non-overlapping 'peaks' in the occurrence of alleles were apparent. Once common alleles were recognised, rare alleles of a similar size were binned with them for further analysis. While this method reduced the variation scorable at this marker, it greatly increased confidence in assignment to allele size bins.

Data analysis. Allele frequencies and observed and expected heterozygosities were calculated using GENEPOP 3.0 (Raymond & Rousset 1995). All loci were tested for linkage disequilibrium, and departures from Hardy-Weinberg expectations were assessed for each locus and each sample using the Markov chain method of Guo & Thomson (1992). Allele frequency differentiation between all pairs of samples was tested using Fisher's exact test in GENEPOP 3.0, and overall significance was calculated using the MULTI-P program (J. Mork, University of Trondheim, Norway, unpubl. data). This program takes as input any number of single p-values from independent tests of the same null hypothesis, and calculates that overall probability of equal or worse fit to their common null hypothesis. The test is equivalent to Fishers omnibus test, but uses a Z-score approximation instead of a chi-squared score. The Bonferroni procedure (Rice 1989) was used to correct for multiple tests using a global significance level of 0.05. The program DISPAN (Ota 1993) was used to calculate Nei's D_A (Nei et al. 1983) and to construct a neighbour joining dendrogram (Saitou & Nei 1987).

The proportion of variation between populations was estimated by calculation of fixation indices based on the infinite allele model (IAM) (Kimura & Crow 1964). This model was chosen over the alternative stepwise mutation model (SMM) (Kimura & Ohta 1978) because of the nature of the loci used in this study. Many authors have demonstrated that the SMM is more appropriate for analysis of data from perfect repeat loci (e.g. Colson & Goldstein 1999, Balloux & Goudet 2002). It was clear from the original sequence data on these loci (Dillane et al. 2000) and the extensive analysis carried out in the present study that the loci used were largely imperfect repeats, thus the IAM was more applicable. Based on the IAM, F_{ST} values (Wright 1951) were estimated across populations (Weir & Cockerham 1984) using the program F-STAT (Goudet 1995). The F_{ST} (Wright's fixation index) significance values for each locus were determined by bootstrapping over samples, and significance values for all loci were calculated by jack-knifing over loci (Weir 1990). Significance values for F_{ST} were determined using 1000 permutations and bootstraps for all sample-pairwise comparisons, and the Bonferroni procedure was applied to correct for multiple tests.

RESULTS

Genetic diversity within samples

Extremely high levels of variability were observed at all loci (Table 1), and within samples, mean observed heterozygosity per locus ranged from 0.82 to 0.91. Departures from Hardy-Weinberg equilibrium occurred at Tebl32 in the Algarve sample and at Tebl25 in the South African sample (Table 1). No consistent deviations occurred over either samples or loci, so it was assumed that there was no strong Wahlund effect (whereby there is a mixture of different populations of different gene frequencies in one sample), or major incidence of null alleles. No significant linkage disequilibrium was observed between any pairs of loci. Allele frequencies (not shown here) are available on request from the authors.

Genetic differentiation and population structuring in the east Atlantic and Mediterranean

Fisher's exact tests of genetic differentiation, calculated from allele frequency data, indicate substantial differences between Mauritanian and South African samples and between these and all European samples (Table 2). There was also evidence of more subtle differentiation between the Mediterranean and Atlantic European samples, with the exception of the Galician coast, although none of these values remained significant after correction.

Overall F_{ST} among samples was 0.014 ($p < 0.001$). This value was largely attributable to differences associated with the Mauritanian and South African samples. Among European samples the F_{ST} estimate was considerably smaller at 0.002, although this value was significantly different from zero ($p < 0.05$). Pairwise F_{ST} values were calculated (Table 3), with the highest being observed between the west Mediterranean and both the Mauritanian and South African samples. All comparisons between African and European samples were significantly different from zero ($p < 0.001$). Significant F_{ST} values, prior to Bonferroni correction, were also ob-

Table 1. *Todaropsis eblanae.* Levels of genetic variability observed at 4 microsatellite loci and 1 minisatellite locus within samples examined in this study. N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; p-value, probability of departure from Hardy-Weinberg expectations. Values remaining significant after tablewide correction using the sequential Bonferroni procedure given in bold

	West of Ireland	English Channel	Galician coast	Algarve	West Mediterranean	Mauritania	South Africa	Mean across samples
Tebl20								
N_A	12.0	13.0	15.0	13.0	16.0	14.0	19.0	14.6
H_O	0.86	0.90	0.88	0.85	0.83	0.97	0.81	0.87
H_E	0.84	0.91	0.90	0.88	0.84	0.89	0.87	0.88
p-value	0.728	0.787	0.332	0.490	0.900	0.082	0.184	0.468
Tebl32								
N_A	9.0	9.0	9.0	9.0	11.0	9.0	11.0	9.6
H_O	0.88	0.77	0.86	0.80	0.79	0.91	0.83	0.83
H_E	0.86	0.84	0.84	0.85	0.84	0.86	0.83	0.85
p-value	0.773	0.438	0.360	**0.007**	0.070	0.046	0.732	0.019
Tebl812								
N_A	12.0	13.0	15.0	14.0	14.0	20.0	22.0	15.7
H_O	0.88	0.80	0.87	0.87	0.89	0.90	0.90	0.87
H_E	0.88	0.88	0.89	0.89	0.88	0.89	0.94	0.89
p-value	0.418	0.335	0.645	0.079	0.567	0.820	0.206	0.344
Tebl25								
N_A	21.0	17.0	22.0	18.0	19.0	24.0	26.0	21.0
H_O	0.98	0.90	0.98	0.95	0.92	0.93	0.82	0.93
H_E	0.92	0.93	0.92	0.97	0.92	0.90	0.94	0.93
p-value	0.564	0.384	0.974	0.467	0.232	0.795	**0.000**	0.166
Teblmini								
N_A	27.0	27.0	29.0	24.0	28.0	27.0	27.0	27.0
H_O	0.81	0.97	0.95	0.86	0.90	0.92	0.93	0.91
H_E	0.94	0.93	0.96	0.94	0.95	0.94	0.95	0.94
p-value	0.012	0.469	0.530	0.278	0.249	0.647	0.044	0.030

Table 2. *Todaropsis eblanae.* Results of Fisher's exact probability tests of differences in allele frequencies between all samples. Above diagonal: multilocus probabilities of homogeneity. Below diagonal: individual loci exhibiting significant differences (1, Tebl20; 2, Tebl32; 3, Tebl812; 4, Tebl25; 5, Teblmini; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Values remaining significant after correction for 0.05 significance level using the sequential Bonferroni procedure are given in bold. NS: not significant

	West of Ireland	English Channel	Galician Coast	Algarve	West Mediterranean	Mauritania	South Africa
West of Ireland	–	0.220	0.130	0.432	0.052	<0.001	<0.001
English Channel	1*	–	0.944	0.730	0.100	<0.001	<0.001
Galician coast	NS	NS	–	0.685	0.775	<0.001	<0.001
Algarve	NS	NS	NS	–	0.172	<0.001	<0.001
West Mediterranean	3**	3*	NS	3*	–	<0.001	<0.001
Mauritania	**1*** 2*** 4*** 5****	**1*** 2*** 4* 5****	**1*** 2*** 3*** 4*** 5*****	**1*** 2*** 3** 4* 5*****	**1*** 2*** 3*** 4*** 5*****	–	<0.001
South Africa	**1*** 2* 3* 4*****	3* **4*****	1* 2** **3*** 4*****	**1*** 2* 3** 4*	**1*** 2*** 3*** 4*****	**1*** 2*** 4*	–

served between some Atlantic and Mediterranean samples (Table 3). Within north Atlantic waters the only notable value was between the Irish sample and the Galician coast, but this marginally significant value did not remain so after correction for multiple tests.

Measures of Nei's D_A also demonstrated that the Mauritanian and South African samples are the most genetically distinct populations (Table 3). The distance values for South Africa and all other samples are lower than those for Mauritania and the Euro-

Table 3. *Todaropsis eblanae*. Below diagonal: pairwise estimates of multilocus F_{ST}, with results of permutation tests showing significant departures from zero (*p < 0.05, **p < 0.01, ***p < 0.001, values remaining significant after sequential Bonferroni correction in bold). Above diagonal: pairwise estimates of Nei's D_A genetic distance

	West of Ireland	English Channel	Galician Coast	Algarve	West Mediterranean	Mauritania	South Africa
West of Ireland	0.089	0.071	0.078	0.076	0.150	0.139	
English Channel	0.003		0.062	0.082	0.085	0.158	0.147
Galician coast	0.004*	0.000		0.063	0.052	0.140	0.117
Algarve	0.000	0.000	0.001		0.081	0.161	0.133
West Mediterranean	0.005*	0.007*	0.001	0.003		0.179	0.159
Mauritania	**0.021*****	**0.017*****	**0.022*****	**0.020*****	**0.033*****		0.114
South Africa	**0.022*****	**0.015*****	**0.016*****	**0.020*****	**0.031*****	**0.012*****	

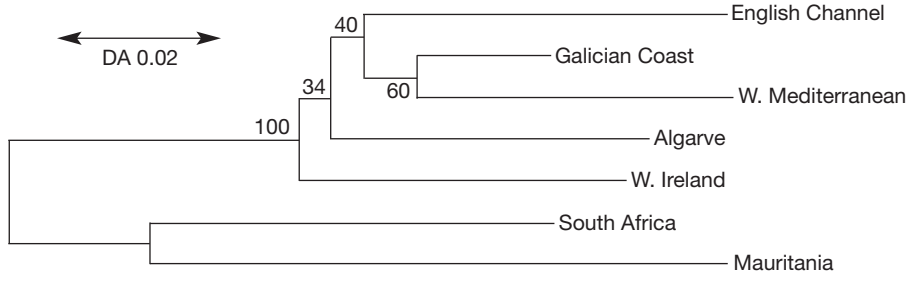

Fig. 2. *Todaropsis eblanae*. Neighbour-joining dendrogram from Nei's D_A genetic distance measures

pean samples, suggesting, as with Fisher's exact tests (Table 2) and F_{ST} values (Table 3), that the Mauritanian sample is more genetically distinct from European samples than the South African sample. The result is also reflected in the neighbour-joining dendrogram (Fig. 2), showing a European and an African cluster.

DISCUSSION

Population structure of *Todaropsis eblanae* in African and European waters

Extremely high levels of genetic variability were observed at all loci used in this study, indicating that, as has been observed in other cephalopod species, microsatellite and minisatellite DNA loci can act as a more sensitive gauge of diversity at the molecular level than less variable markers such as allozymes or mitochondrial DNA (Adcock et al. 1999, Shaw et al. 1999, Murphy et al. 2002). Our findings imply that the South African and Mauritanian populations are genetically distinct, both from one another and from European populations. Furthermore, the Mauritanian sample is more differentiated from European populations than the more geographically distant South African population. This pattern might arise through a range of circumstances. Firstly, it may simply be an artefact of the techniques employed. Secondly, it may reflect historical events affecting the distribution of this species. Thirdly, it may be attributed to the migratory habits in *Todaropsis eblanae*. In regards to the first possibility, the minisatellite and microsatellite loci used in this study can, by their extremely variable nature and high mutation rates, reach a state of homoplasy, whereby 2 alleles converge on the same size as a result of different mutational histories (Angers & Bernatchez 1997). The result is that 2 populations which may appear similar by state are not so by descent. Sequencing of alleles of identical size might clarify this situation.

Alternatively, if we are to assume that the genetic data here reflect true relationships among these populations, then historical events and population dynamics of *Todaropsis eblanae* should be considered. Climatic cycles during the Pleistocene, which apparently led to the regional extinction and geographical displacement of some Atlantic fishes, may have left genetic imprints on the present-day populations of many marine species (Grant & Waples 2000). Steep drops in water temperature at that time would most likely have resulted in fragmentation and displacement of populations, which in the case of *T. eblanae* might have meant a retreat southwards of northern stocks towards equatorial areas, with those

closer to the equator (e.g. Mauritania) being displaced towards the west Atlantic. Hastie et al. (1994) have shown that *T. eblanae* may change its distribution in response to changing environmental conditions, as suggested by unusually large numbers, associated with hydrographic anomalies, occurring in the North Sea in 1987. Post-glacial recolonisation to the full range in the east Atlantic could then have led to the patterns of variation observed in the present study, with Mauritanian squid representing one glacial refuge and South African and Europeans representing others. This might also explain the occasional records of *T. eblanae* in the west Atlantic (Lipinski et al. 1992).

Regarding the movements of *Todaropsis eblanae*, it seems unlikely that any gene flow could occur between South African and European populations. This species is short-lived, and is not known to undertake major migrations which could facilitate interbreeding of these groups. In fact, as suggested by Lordan (2001), this species is probably the least mobile of the ommastrephid squids in terms of migratory habits and is more likely to behave like the neritic loliginid squid species, some of which have been shown to exhibit significant population differentiation across their range (Shaw et al. 1999).

Genetic differentiation within European waters

Subtle differentiation between Mediterranean and European samples was evident from the overall F_{ST} value (0.002), which was statistically significant ($p < 0.05$). Pairwise heterogeneity tests, as well as pairwise F_{ST} and genetic distance measures suggested that differentiation was largely attributable to the Mediterranean sample. Genetic segregation of Mediterranean and Atlantic stocks might occur as a result of both the Straits of Gibraltar and the Almeria–Oran oceanographic front (in the western Mediterranean) acting as barriers to dispersal. This is likely the case in a number of species of both fish and cephalopods (Lundy et al. 1999, Naciri et al. 1999, Bahri-Sfar et al. 2000, Dillane 2001). Conversely, there is evidence to suggest that movement between the Mediterranean and Atlantic occurs in some species of finfish and shellfish, such as swordfish (Kotoulas et al. 1995), the European anchovy (Magoulas et al. 1996) and lobsters (Stamatis et al. 2004). Further sampling of *Todaropsis eblanae* within its range in the Mediterranean would provide a better assessment of population structure and help to calibrate observed differences between the Mediterranean and Atlantic waters. It must also be emphasised that observed differentiation was associated with low measures of genetic distance and marginally significant F_{ST} estimates, and it has been demonstrated that when using highly variable nuclear loci (such as microsatellites), the relationship between statistical and biological significance may be weak due to the high levels of heterozygosity associated with such markers (Hedrick 1999).

There was an obvious lack of discernable genetic differentiation within *Todaropsis eblanae* of European Atlantic waters. While migratory activity in *T. eblanae* is not well understood, the appearance and subsequent absence of this species in an area as far north as the coast of Scotland (Hastie et al. 1994) indicates that these squid move over relatively large distances when conditions are suitable. If gene flow is maintained through periodic dispersal of individuals, identification of what are potentially discrete genetic stocks is made more difficult, even with rapidly mutating markers such as microsatellites and minisatellites. Furthermore, while this species may be less migratory than other ommastrephid species, there is substantial dispersal potential at the planktonic larval stage. The application of a larger suite of microsatellite loci might clarify patterns of variability in this area.

At present, cephalopods remain non-quota species (Anonymous 2003) but as a group they will come under increased pressure as fin-fish resources are depleted and the fishing industry seek out new resources. While the situation in European waters remains unclear, our results suggest there are 3 genetically distinct populations which should be managed as separate stocks. In the case of the west African fishery (one of the largest for *Todaropsis eblanae*) an intensive spatio-temporal study using these, and additional molecular markers, should be undertaken to determine the extent of this group and where it is separated from the European stocks. Such a study might allow further insights into the evolution of this population structure and the barriers to gene flow between groups, whether historical or due to present oceanographic and environmental conditions. This in turn will allow fisheries management groups to make informed decisions regarding the management of this resource.

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LITERATURE CITED

Adcock GJ, Shaw PW, Rodhouse PG, Carvalho GR (1999) Microsatellite analysis of genetic diversity in the squid *Illex argentinus* during a period of intensive fishing. Mar Ecol Prog Ser 187:171–178

Angers B, Bernatchez L (1997) Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Mol Biol Evol 14(3):230–238

Anonymous (2003) Report of the Working Group on Cephalopod Fisheries and Life History. Living Resources Committee, ICES 2003/G:02. www.ices.dk/reports/LRC/2003/WGCEPH/wgceph03.pdf

Arkhipkin AL, Laptikhovsky VV (2000) Age and growth of the squid *Todaropsis eblanae* (Cephalopoda Ommastrephidae) on the north-west African shelf. J Mar Biol Assoc UK 80:747–748

Bahri-Sfar L, Lemaire C, Ben Hassine OK, Bonhomme F (2000) Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proc R Soc Lond B 267:929–935

Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. Mol Ecol 11:771–783

Brierley AS, Thorpe JP, Pierce GJ, Clarke MR, Boyle PR (1995) Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic Ocean. Mar Biol 122:79–86

Caddy JF, Rodhouse PG (1998) Cephalopod and groundfish landings: evidence for ecological change in global fisheries? Rev Fish Biol Fish 8:431–444

Carvalho GR, Thomson A, Stoner AL (1992) Genetic diversity and population differentiation of the shortfin squid (*Illex argentinus*) in the south-west Atlantic. J Exp Mar Biol Ecol 158:105–121

Chikhi L, Bonhomme F, Agnese JF (1998) Low genetic variability in a widely distributed and abundant clupeid species, *Sardinella aurita*: new empirical results and interpretations. J Fish Biol 52:861–878

Colson I, Goldstein DB (1999) Evidence for complex mutations at microsatellite loci in Drosophila. Genetics 152: 617–627

Dillane E (2001) Population genetics and molecular systematics of the ommastrephid squid species, *Illex coindetii* (Verany, 1839) and *Todaropsis eblanae* (Ball, 1841). PhD thesis, National University of Ireland, Cork

Dillane E, Galvin P, Coughlan J, Rodhouse PGK, Cross TF (2000) Polymorphic variable number of tandem repeat (VNTR) loci in the ommastrephid squid, *Illex coindetii* and *Todaropsis eblanae*. Mol Ecol 9:1002–1004

Garthwaite RL, Berg CJ jnr, Harrigan J (1989) Population genetics of the common squid *Loligo pealei*, LeSueur, 1821, from Cape Cod to Cape Hatteras. Biol Bull 177:287–294

Gonzalez AF, Rasero M, Guerra A (1994) Preliminary study of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) in northern Spanish Atlantic waters. Fish Res 21:115–126

Goudet J (1995) F-STAT (1.2), a program for IBM compatible PCs to calculate Weir and Cockerham's (1984) estimators of F-statistics. J Hered 86:485–486

Grant WS, Waples RS (2000) Spatial and temporal scales of genetic variability in marine and anadromous species: Implications for fisheries oceanography. In: Harrison PJ, Parsons TR (eds) Fisheries oceanography: an integrative approach to fisheries ecology and management. Blackwell Science, Oxford, p 61–93

Guo SW, Thomson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361–372

Hastie LC, Joy JB, Pierce GJ, Yau C (1994) Reproductive biology of *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) in Scottish waters. J Mar Biol Assoc UK 74:367–382

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53(2):313–318

Hernandez-Garcia V (2002) Reproductive biology of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) off northwest Africa (4N, 35N). Bull Mar Sci 71: 347–366

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–738

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc Natl Acad Sci USA 75:2868–2872

Kotoulas G, Magoulas A, Tsimenides N, Zouras E (1995) Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish, *Xiphias gladius*. Mol Ecol 4:473–481

Lipinski MR (1992) Cephalopods and the Benguela ecosystem, trophic relationships and impact. S Afr J Mar Sci 12: 791–802

Lipinski MR, Payne AIL, Rose B (1992) The importance of cephalopods as prey for hake and other groundfish in South African waters. In: Payne AIL, Brink KH, Mann KH, Hilborn R (eds) Benguela trophic functioning. S Afr J Mar Sci 12:651–662

Lipinski MR, Roeleveld MAC, Underhill LG (1993) Comparison of the statoliths of *Todaropsis eblanae* and *Todarodes angolensis* (Cephalopoda: Ommastrephidae) in South African waters. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo, p 263–273

Lordan C (2001) Investigations into the fisheries and biology of ommastrephid squid species in Irish waters. PhD thesis, Aquaculture Development Centre, Department of Zoology and Animal Ecology, Cork, National University of Ireland

Lordan C, Burnell GM, Cross TF (1998) The diet and ecological importance of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) in Irish Waters. S Afr J Mar Sci 20:153–163

Lundy CJ, Moran P, Rico C, Milner RS, Hewitt GM (1999) Macrogeographical population differentiation in oceanic environments: a case study of European hake (*Merluccius merluccius*), a commercially important fish. Mol Ecol 8: 1889–1898

Magoulas A, Tsimenides N, Zouras E (1996) Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European anchovy (*Engraulis encrasticolus*). Mol Biol Evol 13(1):178–190

Murphy JM, Balguerias E, Key LN, Boyle PR (2002) Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the northwest African coast. Bull Mar Sci 71:545–553

Naciri M, Lemaire C, Borsa P, Bonhomme F (1999) Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). J Hered 90:591–596

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 19:153–170

Nesis KN (1987) Cephalopods of the world. TFH Publications, Neptune City, NJ

Nigmatullin CM (2002) Preliminary estimation of total stock size and production of ommastrephid squids in the world ocean. Bull Mar Sci 71:1134

O'Connell M, Dillon MC, Wright JM, Bentzen P, Merkouris S, Seeb J (1998) Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. J Fish Biol 53:150–163

Ota T (1993) DISPAN: genetic distance and phylogenetic analysis. Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, PA www.bio.psu.edu/People/Faculty/Nei/Lab/Programs.html

Perez-Losada M, Guerra A, Carvalho GR, Sanjuan A, Shaw PW (2002) Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian peninsula indicated by microsatellite DNA variation. Heredity 89(6):417–424

Rasero M (1994) Relationships between abundance and upwelling: the case of *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) in Galician waters (NW Spain). ICES Coun Meet K:20

Rasero MP (1996) Short-finned squid *Todaropsis eblanae* (Ball, 1841) (Cephalopoda, Ommastrephidae): Life cycle and ecological aspects in the coastal waters of Galicia (NW Iberian peninsula). PhD thesis, University of Santiago de Compostela

Rasero M, Gonzalez AF, Castro BG, Guerra A (1996) Predatory relationships of two sympatric squid, *Todaropsis eblanae* and *Illex coindetii* (Cephalopoda: Ommastrephidae) in Galician waters. J Mar Biol Assoc UK 76:73–87

Raymond M, Rousset F (1995) GENEPOP (Version 1.2) — population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analysing tables of statistical tests. Evolution 43:221–225

Robin JP, Denis V, Royer J, Challier L (2002) Recruitment, growth and reproduction in *Todaropsis eblanae* (Ball, 1841), in the area fished by French Atlantic trawlers. Bull Mar Sci 71:711–724

Roper CFE, Sweeney MJ, Nauen CE (1984) FAO species catalogue, Vol 3. Cephalopods of the world: an annotated and illustrated catalogue of species of interest to fisheries. Fisheries Synopsis. FAO 125(3):1–277

Saitou N, Nei M (1987) The neighbour-joining method: a new methodology for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shaw PW (2002) Past, present and future applications of DNA based markers in cephalopod biology. Workshop report. Bull Mar Sci 71:67–78

Shaw PW, Pierce GJ, Boyle PR (1999) Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Mol Ecol 8:407–417

Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2004) Mitochondrial DNA variation in northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. Mol Ecol 13(6):1377–1390

Weir BS, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Weir BS (1990) Genetic data analysis. Sinauer Associates, Sunderland, MA

Winnepenninckx B, Backeljau T, Dewachter R (1993) Extraction of high molecular weight DNA from mollusks. Trends Gen 9:407

Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354

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