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BRIEF COMMUNICATIONS

Population genetics of invasive common carp *Cyprinus carpio* L. in coastal drainages in eastern Australia

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The common carp *Cyprinus carpio* introduced in two drainages in eastern Australia are largely descended from European common carp, and in a third drainage they descend largely from East Asian common carp. The partial genetic differentiation among the species in those drainages is consistent with their origins.

Key words: freshwater fish; genetic structure; invasive species; koi; microsatellite; Prospect Reservoir.

Freshwater environments are fundamentally discontinuous, with adjacent drainages being isolated from each other by land or salt-water barriers. Over geological time, obligate freshwater fish can disperse between adjacent drainages through river capture, connection of rivers when sea levels are low and connection of rivers that drain into salt lakes when periods of increased rainfall freshen these lakes. Dispersal can also occur over much shorter time frames through connection of river headwater, extreme weather conditions (*e.g.* water spouts) picking up and transporting fish, accidental movement by terrestrial animals (*e.g.* eggs sticking to the feathers and feet of waterfowl) and floods either directly joining drainages or allowing organisms to disperse through flood plumes (Unmack, 2001). The rivers in the eastern margin of Australia are characteristically short and fast flowing, have high initial gradients, and drain directly into the Pacific Ocean (Jerry, 2008). These rivers support a unique assemblage of freshwater fish that follow a clinal distribution among drainages, with species' presence or absence being determined largely by climate (Unmack, 2001). Population genetic studies have revealed genetic structuring and the presence of evolutionary significant units (ESU) and sub-species across different drainages (Page *et al.*, 2004; Faulks *et al.*, 2008; Jerry, 2008). Studies have also identified genetic

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similarities between fish populations in adjacent rivers that are joined in times of flood (Wong *et al*., 2004; Knight *et al*., 2009). The climate-driven, clinal distribution of species across the drainages in eastern Australia suggests that there are few distinct barriers to dispersal and little long-term isolation between drainages (Unmack, 2001). This lack of strong barriers to dispersal is further supported by a recent population genetic investigation of the duckbill platypus *Ornithorhynchus anatinus*, an aquatic mammal native to Australia, which indicated that this species disperses both along river channels and overland between river basins (Kolomyjec *et al*., 2009).

Since European colonization, non-indigenous fish species have been introduced into drainages throughout Australia. One of the most successful of these introduced species is the common carp *Cyprinus carpio* L., which has been introduced into Australia on several occasions since the late 19th century (Clements, 1988; Koehn *et al*., 2000) and has spread from the introduction sites through a combination of natural range expansion and intentional and accidental release (Koehn *et al*., 2000). *Cyprinus carpio* is now the dominant species in many parts of the Murray–Darling Basin (MDB) (Lintermans, 2007), the largest river basin in the Australian continent, and it is also found in many smaller river basins in the south-west, south-east and eastern coast of the continent (Koehn, 2004). The population genetics of *C. carpio* in the MDB has been investigated extensively, with four strains, koi, Prospect, Boolara and Yanco, confirmed to have been introduced, and significant genetic structuring detected between sub-drainages (Shearer & Mulley, 1978; Mulley & Shearer, 1980; Davis *et al*., 1999; Haynes *et al*., 2009). The connectivity and history of *C. carpio* has not previously been investigated, however, in any drainage along the eastern coast of Australia. In this study, the population genetics of introduced *C. carpio* from three coastal drainages and one dam in eastern Australia is investigated to determine the extent to which the *C. carpio* from the three drainages have a common origin and have become genetically differentiated.

The drainages on the east coast of Australia that were sampled were the Hunter River at a site close to Clarence Town (CT), and the Hawkesbury–Nepean (HN) and the Parramatta (PM) Rivers, which run through or run adjacent to urban areas of Sydney (Fig. 1). In addition, samples were collected from Prospect Reservoir (PR), which is located in the outer suburbs of Sydney and was one of the first introduction sites for *C. carpio* in eastern Australia (Stead, 1929) (Fig. 1). Specimens were collected by electrofishing between November 2004 and June 2006, with 27, 26, 20 and 24 fish sampled from the CT, HN, PM and PR sites, respectively. A fin clip was taken from each individual and immediately placed in 70% ethanol. The Inland Fisheries Department in Berlin, Germany, also donated samples of wild *C. carpio* from the River Danube ($n = 29$) and Japanese koi carp ($n = 30$). Another 39 Japanese koi carp were donated by a fish farm in Sydney. DNA extraction and genotyping of 14 microsatellite loci followed the method of Haynes *et al*. (2009).

GENEPOP 1.2 (Raymond & Rousset, 1995) was used to test for significant departures from Hardy–Weinberg expectations (HWE) at each sample site, for significant differences in alleles frequencies between each sample site (Fisher's exact test) and to calculate F_{ST} (Weir & Cockerham, 1984) between each pair of sample sites. Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992), which partitions genetic variation within and among populations, was performed in GENALEX 6.0 (Peakall & Smouse, 2006), and significance was assessed with the Φ_{PT} statistic against an empirical null distribution derived from 9999 random permutations of the data.

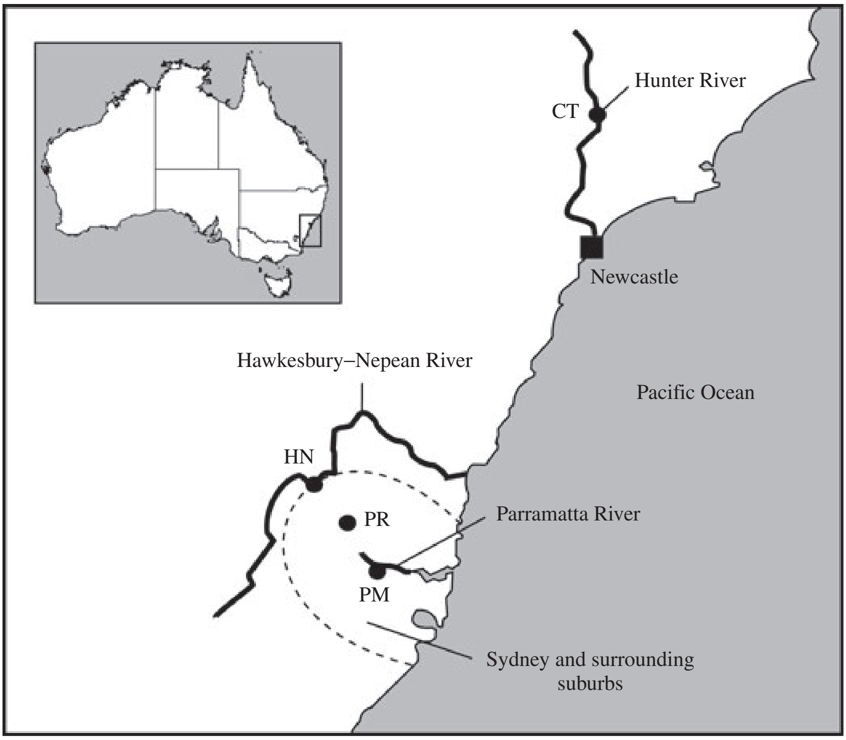

FIG. 1. Sampling locations of *Cyprinus carpio* in eastern Australia waterways. HN = Hawkesbury–Nepean River (33·60297° S; 150·80724° E), PM = Parramatta River (33·80727° S; 151·00468° E), CT = Hunter River (32·58416° S; 151·783503° E). The source of the Prospect strain is also shown: PR = Prospect Reservoir (33·815° S; 150·901° E).

To determine the extent to which the sampled *C. carpio* have a common origin, all samples were analysed using STRUCTURE 2.1 (Pritchard *et al.*, 2000). STRUCTURE implements a Bayesian clustering analysis that assigns individuals into a predetermined number of clusters (K) under the assumptions of HWE and linkage equilibrium. The estimated proportion of ancestry from any given cluster (K) in each individual is given by the statistic q_K. Individuals can be assigned 100% to a single cluster ($q_K = 1{\cdot}0$) or partially to two or more clusters (*i.e.* individuals with mixed ancestry). In accordance with Vähä & Primmer (2006), individuals assigned 90–100% to a single cluster ($q_K = 0{\cdot}9$–$1{\cdot}0$) were considered to have ancestry solely from that cluster, while individuals attributed <90% to one cluster ($q_K < 0{\cdot}9$), with the balance made up from another cluster, were considered to have mixed ancestry. Run conditions were 500 000 burn-in steps and 1 000 000 Markov-Chain Monte-Carlo steps, under the admixture and allele frequencies correlated models. The analysis was run for $K = 1$–10 clusters, with four iterations for each value of K to check for consistency between runs. The ΔK statistic (Evanno *et al.*, 2005) was used to determine the actual number of populations present. This statistic measures the rate of change of the log probability of data between successive values of K. When plotted against K, ΔK produces a peak at the value of K approximating to the true value number of the populations present.

The samples from the CT, PM and PR sites showed significant departures from HWE ($P = 0{\cdot}0047$ for CT and $P < 0{\cdot}0001$ for PM and PR), while genotype frequencies in the HN sample were consistent with HWE frequencies ($P = 0{\cdot}7802$). Significant departure ($P < 0{\cdot}0001$) from panmixia was detected between each pair of sample sites. F_{ST} values between sites were 0·0918 (CT and HN), 0·1964 (HN and PM), 0·1783 (CT and PM), 0·0597 (PR and HN), 0·1505 (PR and CT) and 0·2985 (PR and PM). The AMOVA analysis was highly significant ($P = 0{\cdot}0001$), partitioning 25% of the genetic variation among sites and the remainder (75%) within sites.

In the assignment testing performed in STRUCTURE 2.1, two peaks were produced in the graph of ΔK against K, with the largest peak at $K = 2$ and a smaller peak at $K = 4$ (data not shown). At $K = 2$, the *C. carpio* from the River Danube and both samples of Japanese koi carp were partitioned almost exclusively into the two clusters, with 96% of the overall genetic variation of the koi carp assigned to the first cluster and 95% of the overall genetic variation of the River Danube *C. carpio* assigned to the second cluster (Fig. 2). The *C. carpio* from the CT, HN and PR sites were largely assigned to the same cluster as the River Danube *C. carpio*. Two individuals from HN and nine from CT were partially assigned to the same cluster as the koi carp, with individuals assigned between 10·2 and 40·1% ($q_K = 0{\cdot}102$–$0{\cdot}401$) to this cluster. All *C. carpio* from the PM site were at least partially assigned to the same cluster as the koi carp, with individual genotypes assigned between 18·6 and 98·7% ($q_K = 0{\cdot}186$–$0{\cdot}987$) to this cluster.

In the assignment analysis at $K = 4$ clusters (Fig. 2), koi carp from Germany and koi carp from a fish farm in Australia were separated into two clusters, with *C. carpio*

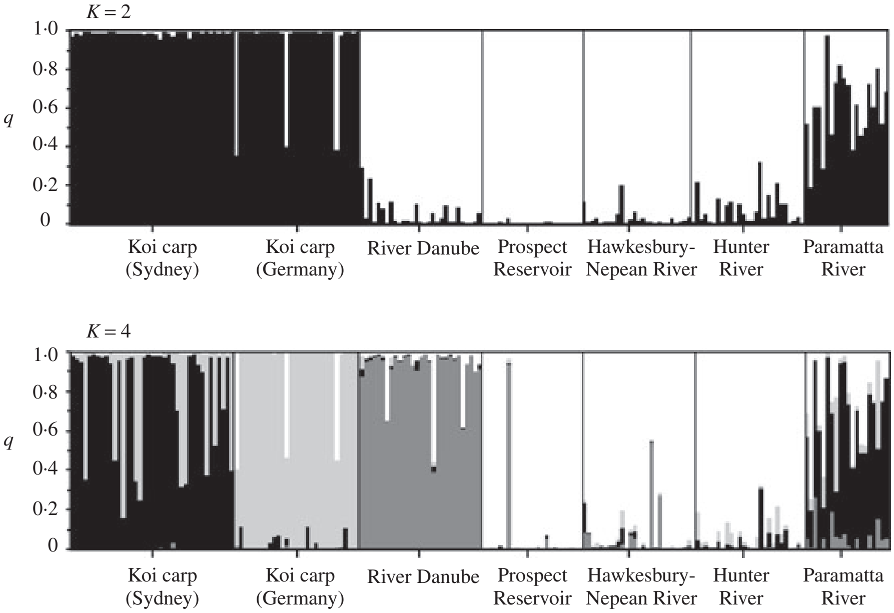

FIG. 2. Assignment analysis in STRUCTURE for $K = 2$ (top) and $K = 4$ (bottom) clusters. Individual assignment to each cluster (q) is indicated by different shades (black and white for the $K = 2$ analysis; black, white, pale grey and dark grey for the $K = 4$ analysis).

from the River Danube largely assigned to a third cluster. *Cyprinus carpio* from the CT, HN and PR sites were largely assigned to a fourth cluster. Consistent with the $K = 2$ analysis, some *C. carpio* from the CT and HN sites were also partially assigned to the same clusters as the koi carp, with individual assignments between 11·6 and 32·0% ($q_K = 0{\cdot}116–0{\cdot}320$) to the two clusters associated with the koi carp (clusters pooled for this calculation). All *C. carpio* from the PM site were at least partially assigned to the same pooled koi cluster ($q_K = 0{\cdot}247–0{\cdot}993$), with the rest of the genotypes largely assigned to same cluster as *C. carpio* from the PR, HN and CT sites.

There is much concern that *C. carpio* will further expand their range within Australia, with a consequential increase in the uprooting of aquatic vegetation, undermining of river banks, increased water turbidity and algal blooms, and decline in indigenous species (Koehn *et al*., 2000; Angeler *et al*., 2002; Parkos *et al*., 2003; Tapia & Zambrano, 2003; Pinto *et al*., 2005). Aside from natural mechanisms of dispersal, active movement by people can rapidly move *C. carpio* between drainages that might otherwise be largely isolated. Active movement can arise by intentional release to create coarse fisheries, accidental release when *C. carpio* fry are used as live bait by fisherman and subsequently discarded, and contamination of stocks of native fish with *C. carpio* fry in restocking programmes (Koehn *et al*., 2000). In addition, koi carp, a strain of *C. carpio* originating from Japan that have been selectively bred for ornamental colouration, are kept as pets and sometimes released into drainages (Koehn *et al*., 2000; Haynes *et al*., 2009).

The results of this study demonstrate partial genetic differentiation among *C. carpio* populations in the Hawkesbury–Nepean, Parramatta and Hunter Rivers. As these populations are isolated by land barriers, they will probably retain their differentiation in the immediate future, barring active movement of *C. carpio* by humans. Long-term migration and gene flow are likely if populations persist, however, as native freshwater species show wide distributions across different drainages in eastern Australia (Unmack, 2001).

The study also reveals the pattern of colonization of the four drainages investigated. In the assignment analyses in STRUCTURE, the two peaks produced at $K = 2$ and $K = 4$ clusters in the graph of ΔK against K likely stem from the presence of hierarchical structuring. In the $K = 2$ analysis, the two clusters probably correspond with the European common carp sub-species, *Cyprinus carpio carpio*, and the East Asian sub-species, *Cyprinus carpio haematopterus* (Zhou *et al*., 2003; Kohlmann *et al*., 2005), with the *C. c. carpio* and *C. c. haematopterus* sub-species being represented by carp from the River Danube and the farmed koi carp, respectively. This analysis indicates that all *C. carpio* from Prospect Reservoir and most from the Hawkesbury–Nepean and Hunter Rivers have exclusively European ancestry, as these fish are assigned almost exclusively to the same cluster as *C. carpio* from the River Danube. This study also reveals that koi carp are the dominant strain in the Parramatta River, with all fish showing at least some koi ancestry ($q_{koi} > 0{\cdot}1$) and one individual having exclusively koi ancestry ($q_{ko\mathrm{i}} > 0{\cdot}9$). This differentiation between the Parramatta River, on one hand, and the Hawkesbury–Nepean and Hunter Rivers, on the other, is reinforced by the F_{ST} values among the three river sites.

The finding that the European-descended *C. carpio* sampled in eastern Australia grouped into a single cluster rather than into three or four distinct clusters suggests a common origin. In the $K = 4$ assignment analysis, the clusters representing the *C. c. carpio* and *C. c. haematopterus* sub-species are further divided: koi carp from the German and Australian fish farms are partitioned into separate clusters and the River Danube *C. carpio* and European-descended *C. carpio* from eastern Australia are assigned largely into separate clusters. The cluster to which the *C. carpio* from Australia are largely assigned probably represents the Prospect strain of carp. This strain is known to have been founded from some 15 fingerlings of unknown origin in 1907–1908 in trout runs close to Prospect Reservoir (Stead, 1929) and later released into the Reservoir and used to seed other populations around the Sydney Basin (Clements, 1988). It is likely that Prospect strain *C. carpio* from the Sydney Basin were translocated to the Hunter River, although whether this was in a single step or through a series of rivers along the east coast being progressively seeded with *C. carpio* is unknown. The Prospect and koi carp are also clearly interbreeding, as the majority of *C. carpio* from the Parramatta River show ancestry from both strains (Fig. 2).

Irresponsible release of koi carp is probably still occurring, as indicated by the high prevalence of koi carp in the Parramatta River and the presence of individuals with koi ancestry in the Hawkesbury–Nepean and Hunter Rivers. Genetic studies of indigenous *C. carpio* populations in Europe, Vietnam and Japan have similarly indicated that strains introduced from other parts of the world have escaped into local drainages and have interbred with *C. carpio* already present (Balon, 2004; Kohlmann *et al*., 2005; Thai *et al*., 2006; Mabuchi *et al*., 2008). As koi carp are a popular aquarium and pond fish in some parts of Australia, their release could readily introduce *C. carpio* into new drainages, add additional genetic variation to already established populations and confound eradication attempts. As this study suggests that koi carp have been released and are interbreeding with existing strains, and subsequently dominating some drainages, there is a strong case for banning the importation and keeping of all varieties of *C. carpio* from Australia altogether, including koi carp, to further protect aquatic environments from invasive species.

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