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Failure of microsatellite's cross-species amplification in common ground beetle *Pterostichus melanarius* (Illiger)

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In the present paper, we describe results of cross-species amplification of microsatellite loci in wing dimorphic carabid *Pterostichus melanarius* - model species for diverse ecological studies. In particular wing dimorphism determined genetically, with simple Mendelian fashion and discovery of brachyptery gene provides unique opportunities to study gene flow within metapopulation. One hundred individuals originating form nine island populations of Mamry Lake archipelago were studied. Using PCR primers from five different species of Carabidae, especially including closely related species – *Pterostichus oblongopunctatus* we failed to establish firm ground of population genetic tool for the beetle. We review works on cross-species amplification in invertebrates and show that success of cross-species strategy among coleopteran species from different genus is rather highly doubtful. We present procedures and results that that might be of importance for ecologists tempted by scientific opportunities given by *P. melanarius*.

Key words: Pterostichus melanarius, Coleoptera, Carabidae, cross-species amplification.

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INTRODUCTION

There are many good "model species", that pose unique features, which help to explain particular scientific questions. For example laboratory mice, *Drosophila*, or *Daphnia* proved their importance in biological studies. Common ground beetle *Pterostichus melanarius* is another example. There are tens of works inspecting different aspects of its biology (Ericson 1977, David 2000, Symondson *et al.* 2000, Winder *et al.* 2001). Particularly, wing dimorphism make this animal perfect object in studies on invasions and

dispersal (Niemelä & Spence 1991, 1999, Zalewski 2004). Briefly, populations of this common ground beetle contain long winged (macropterous), as well as wingless (brachypterous) individuals. The trait is determined genetically, with simple Mendelian fashion of inheritance and brachyptery gene being a dominant allele (Aukema *et al*. 1996). As consequence of wing type inheritance and emigration fraction of macropterous individuals in population gives good idea about population age (Den Boer *et al.* 1980, Zalewski 2004) – the characteristic rarely studied in natural populations. Additionaly brachyptery gene was discovered (Ober 2002). Thus, *P. melanarius* provides opportunity to study genetic processes in new versus old populations and genetic structure of the species formed by two different phenotypic forms: dispersive and non-dispersive, as well as gene flow in metapopulations. All of them, are presently important questions in population biology (Hanski *et al.* 2004, Ovaskainen *et al.* 2008) and are reasons for conducting this study.

Simultaneously, resolving of such complex population's questions requires utilization of molecular markers, which present high polymorphism on the species level. Microsatellites are one of the most popular genetic marker of this type. They are defined as tandem repeats of short (from two to six nucleotides) DNA motif, forming more or less uniform tracts up to 100 nucleotides long (Chambers & MacAvoy 2000). Microsatellites are found in just about every organisms and organelle and are relatively evenly spaced throughout the genomes (Edwards *et al*. 1991, Stallings *et al*. 1991, Chambers & MacAvoy 2000). High level of polymorphisms interlinked with the power that they provide to solve biological problems, as well as the possibility of analysis using fast and effective technique of PCR cause the microsatellites useful genetic marker for wide range of genetic investigation, ranging from identification of individuals to studies on population level (e.g. Sloane *et al*. 2000, Girman *et al*. 2001, Lee *et al*. 2001, Roeder *et al*. 2001). One of the factors limiting even broader use of microsatellites is that initial identification of the marker is expensive and labour-consuming, moreover requires cloning and sequencing. To overcome these disadvantages, researchers often adapt information about microsatellite markers, originally developed for one (the source) species, for use in other, usually closely related species (the target). This strategy, which is based on using PCR primers described for one species to amplify homologous microsatellite in other species was called cross-species microsatellite amplification and become widely applied, frequently with success (Moore *et al*. 1991, Gemmell *et al*. 1997, Primmer & Ellegren 1998, Gibbs *et al*. 2000). However, application of cross-species microsatellite amplification has been shown to have many limitations. First, the strategy works preferably for species belonging to the same genus or to recently separated genera (Scribner & Pearce 2000). Second, in many cases a given microsatellite may fail to amplify or may be less or even non-polymorphic in target species (Rubinsztein *et al*. 1995, Morin *et al*. 1998). Thus, application of cross-species strategy requires pilot study, which would assess the amplification success of particular markers and their level of polymorphisms in target species, as only polymorphic microsatellites could be successfully used in population genetic studies (Frankham *et al*. 2003).

In the present paper, we describe results of cross-species amplification of microsatellite loci in *Pterostichus melanarius*, using PCR primers from several different species of Carabidae, especially including closely related species – *Pterostichus oblongopunctatus*. Nevertheless our broad cross-species research failed to establish firm ground of population genetic tool for the beetle, we present procedures and results that might be of importance for ecologists using scientific opportunities given by *P. melanarius*. Our basic scientific question was about genetic diversity of young vs. old populations and importance of macropterous vs. brachypterous individuals for gene flow in metapopulation. However, as we fail to study these interesting issues due to problems with amplification they remain to be solved in

future when PCR primers for *Pterostichus melanarius* will be developed.

MATERIAL AND METHODS

Beetles were captured alive on islands and mainland sites on the archipelago and shores of Mamry Lake (coordinates 21'30°–21'52° E, 54'00°–54'10° N). Collected specimens were preserved in 70% ethanol and stored frozen. In total 500 beetles were collected.

Genomie DNA was isolated from 100 individuals originating from 9 different sites. Extraction was performed applying DNeasy® Tissue Kit (Qiagen). Fragments of tissues form abdomens were crushed and suspended in 180 µl of ATL buffer and incubate at 56°C over night with 20 µl of proteinase K (20µg/ml). Following incubation, extraction of DNA followed standard protocol with the DNA being eluted in 100 µl of elution buffer. Results of DNA isolation were checked using electrophoresis technique in 1.5% agaroze gels.

As no microsatellite markers for *P. melanarius* had been described, we applied cross-species amplification strategy. In total we have tested 11 markers. Following primers, amplifying microsatellite sequences in five other Carabidae species were used: Pob1; Pob3–Pob5; Pob10 and Pob14 (source species – *Pterostichus oblongopunctatus*; Lagisz & Wolff 2004), Cins11 and Cins33 (source species – *Carabus insulicola;* Takami & Katada 2001), Cn11/152 (source species – *Carabus nemoralis*, Brouat *et al*. 2002), Cpro98 (source species – *Carabus problematicus*, Gaublomme *et al*. 2003), and Csol6103 (source species – *Carabus solieri*, Garnier *et al*. 2002)

PCR reaction was conducted in volume of 25µl which contained from 1 to 3µl of DNA extract, 10 pmols of each primer and 12,5µl of RedTaq Ready Mix (Sigma), using Biometra thermocycler. Preliminary, PCR reactions were performer under following conditions: 3 min in 95ºC, 35 cycles: 30 s in 94ºC, 45 s in 55ºC, 45 s in 72ºC, 5 min in 72ºC. As no product was obtained amplification of each microsatellite was repeated under different PCR conditions, using gradient of annealing temperatures ranging from 44ºC to 60ºC.

The length of amplified fragments was estimated using CEQ8000 Beckman Coulter automatic sequencer. Data were analyzed using Beckman Coulter Fragment Analysis Software.

RESULTS

Satisfying results, meaning electropherograms, which showed characteristic microsatellite's structure of a peak after analysis of allele's fragment length in sequencing machine – the "stutters bands" and +A peaks were obtained only for Pob10 marker amplified in annealing temperature 55ºC. In all tested individuals (both macropterous and wingless specimens) amplified fragment had exactly the same length (126 base pairs) indicating severe fixation of an allele.

No clear amplification products were visible for other tested loci. Either, complete lack of amplified fragments or multiple "peaks" were obtained in a wide range of annealing temperatures.

DISCUSSION

Our study on cross-species amplification of microsatellite between *Pterostichus oblongopunctatus* (source species) and *P. melanarius* (target species) indicate extremely low success of amplification (16.5%) and complete lack of amplification of polymorphic markers. The observation is rather surprising, as similar strategy on another member of the genus – *Pterostichus quadrifoveolatus* – resulted in amplification of full marker's set. Moreover all of them were polymorphic (Lagisz & Wolff 2004; Table 1). On the other hand, success of cross-species strategy in the case of invertebrates varies substantially among taxa, as well as among species within the same genus (Table 1). For example, utilization of PCR primers amplifying

miecrosatelites in *Carabus punctatoauratus*, in *C. soleri* resulted in high success of amplification with high share of polymorphic loci (Garnier *et al*. 2002), whereas only one marker described for *C. problematicus* was amplified in three different *Carabus* species (Gaublomme *et al*. 2003). Indeed, low efficiency of cross-species strategy in Coleoptera seems not to be rare (Table 1). Similarly, we failed to amplify even single marker in *Pterostichus melanarius* from other Coleopteran species.

In other invertebrate taxa success of amplification seems to be easier to predict. Within the same genus, usually from 40 to 100% of tested loci are amplified with quite substantial portion of polymorphic markers (Table 1). Similar results were obtained for some vertebrate species. For example, in birds, where cross-species strategy is frequently used due to very low frequency of microsatellite sequences in avian genome (Primmer *et al*. 1997), success of amplification usually gives values over 80% with 40-50% of polymorphic markers among amplified ones (Primmer *et al*. 1996; Galbusera *et al*. 2000; Rutkowski *et al*. 2006). As stated by Galbusera *et al*. (2000) either high successful amplification rate and relatively high level of polymorphism might have been interlinked with strict pre-selection of markers, namely testing of markers within genus, however in the case of Coleoptera even such pre-election does not guarantee a success (this study and Table 1). Primmer *et al*. (2005) shown that success of amplification of a locus in cross-species strategy and probability of locus to be polymorphic was higher, when genetic distance between source and target species was small.
This could suggest that genetic distance between *P. melanarius* and *P. oblongopunctatus* is rather high, indicating very ancient separation of their evolutionary lineages. On the other hand, linkage between evolutionary relationship and cross-species of microsatellite loci is not always obvious. As reported by Rutkowski *et al*. (2006), study on woodpecker species showed that homological microsatellites were amplified more efficiently between *Dendrocopos major* and *Jynx torquilla* , than between *Dendrocopos major* and *D. medius*, with the two latest believed to be very closely related.

Success of amplification seems to be also locus-dependent. For example, some loci are possible to amplify in a wide range of species, whereas other are very species-specific. Primmer & Ellegren (1998) showed that *HrU2* locus was amplified and presented polymorphism in such evolutionary distinctive bird groups as woodpeckers and warbrels. Such a locus was also found in Colembolla (Rutkowski *et al*. 2007). On the other hand, Rutkowski *et al*. (2006) found microsatellites which amplified well in wide range of woodpeckers, whereas other were possible to obtain in very limited range of species and rarely exhibit polymorphism. Locus Macu7, described for *Maculinea nausithous*, was cross-amplified only in *M. telesisus*, as a monomorphic, despite testing on three other members of the genus (Zeisset *et al*. 2005). Hence, another explanation of our results could be accidental selection of microsatellite loci with limited capacity to broader utilization. However, this suggestion stays in opposite with results obtained by Lagisz & Wolff (2004) in cross-species test on *P. quadrifoveolatus*. To resolve this problem further studies are required, preferably on wider range of target species.

In summary, we can state that analysed set of markers is not applicable to population studies of *P. melanarius*. Moreover, as shown by our results as well as literature revision, success of cross-species strategy among coleopteran species from different genus is rather highly doubtful. However, some microsatellite loci, which are possible to amplify in wide range of species, can be used as an additional marker in resolving phylogenies or investigating microsatellite sequences evolution. It seems probably, that locus Pob10 (Lagisz & Wolff 2004) could be such a marker. Its amplification, in complete absence of PCR's products for other tested loci suggests, that Pob10 could be also amplified in wider range of coleopteran species.

Table 1. Review of selected examples of cross-species amplification of microsatellite loci in different invertebrate's groups. N – number of loci analysed; SA— success of amplification (number of successfully amplified loci among all tested loci in %); NP – number of polymorphic loci (number of loci with more than one allele detected) ; nr – data not reported

	Source	Target	N	SA	NP	Reference
Coleoptera, Carabidae	*Carabus punctatoauratus*	*C. soleri*	9	89	8	Garnier *at al*. (2002)
	Carabus soleri	*C.punctatoauratus*	11	64	5	
	Carabus problematicus	*C.nemoralis*	6	16.5*	nr	Gaublomme *et al*. (2003)
		C.violaceus	6	16.5*	nr	
		C.auronitens	6	16.5*	nr	
	Pterostichus oblongopunctatus	*P. quadrifovelatus*	6	100	6	Lagisz & Wolff (2004)
Coleoptera, Bruchidae	*Zabrotes subfasciatus*	*Zabrotes sylvestris*	6	50	3	Aebi *et al*. (2004)
	Acanthoscelides obtectus	*A.obvelatus*	6	0	0	Alvarez *et al*. (2004)
		A.argillaceus	6	16.5	1	
Coleoptera, Scolytidae	*Hypothenemus hampei*	*H.seriatus*	7	57	2	Gauthier & Rasplus (2004)
		H.eruditus	7	57	3	
Hymenoptera, Formicidae	*Messor structor*	*Messor barbarus*	7	100	7	Arthofer *et al*. (2005)
		Messor bouvieri	7	43	1	
		Messor maroccanus	7	86	3	
	Formica exsecta	*Formica aquilania*	10	80	nr	Gyllenstrand *et al*. (2002)
		Formica fusca	10	80	nr	
		Formica lugubris	10	80	nr	
		Formica polyctena	10	100	nr	
		Formica sanguinea	10	60	nr	
		Lasius niger	10	50	nr	
		Proformica longiseta	10	60	nr	
		Pogonomyrmex sp.	10	10	nr	
	Formica lugubris	*Formica cinerea*	5	100	1	Chapuisad (1996)
		Formica exsecta	5	100	2	
		Formica uralensis	5	100	4	
		Formica truncorum	5	100	4	
		Formica rufa	5	100	4	
		Formica aquilonia	5	100	3	
		Formica polyctena	5	100	4	
		Formica lugubris	5	100	4	
		Camponotus rufipes	5	40	0	
		Lepthothorax nylanderi	5	20	0	
		Linepithema humilis	5	20	0	
Lepidoptera, Lycaenidae	*Maculinea naustihous*	*Maculinea telesisus*	11	82	8	Zeisset *et al*. (2005)
		Maculinea alcon	11	36	4	
		Maculinea rebeli	11	36	nr	
		Maculinea arion	11	54.5	nr	
	Maculine alcon	*Maculinea telesisus*	1	0	0	
		Maculinea rebeli	1	100	nr	
		Maculinea arion	1	0	0	
		Maculinea naustihous	1	0	0	
Apterygota, Collembola	*Orchesella cincta*	*Orchesella flavescens*	7	86	2	Rutkowski *et al*. (submitted)
	Orchesella villosa	*Orchesella flavescens*	7	57	2	
Hymenoptera, Vespidae	*Polistes annularis*	*Polistes belicosus*	5	40	2	Strassmann *et al*. (1997)
		Polistes dorsalis	5	40	1	
		Polistes dominulus	5	40	1	
	Polistes belicosus	*Polistes annularis*	3	67	0	
		Polistes dorsalis	3	100	2	
		Polistes dominulus	3	33	0	
Molusca	*Patella depressa*	*Patella candei*	11	64	4	Perez *et al*. (2006)
		Patelka rustica	11	45	2	

* – only loci described by authors as presenting interpretable amplification products were included

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