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Original article

Genetic diversity of the red-spotted tokay gecko (*Gekko gecko* Linnaeus, 1758) (Squamata: Gekkonidae) in Southeast Asia determined with multilocus enzyme electrophoresis

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ABSTRACT

Red-spotted tokay geckos, *Gekko gecko*, are distributed mainly in Southeast Asia. They are a traditional Chinese medicine, with the massive hunting for exports dramatically decreasing their numbers. Information on the genetic diversity of these geckos in Southeast Asia is very limited. This study aims to explore intrapopulation and interpopulation genetic variation and the genetic structure of 16 populations collected from different localities in Thailand, Lao People's Democratic Republic, and Cambodia using multilocus enzyme electrophoresis. Relatively high genetic diversity occurred at both the intrapopulation and interpopulation levels. Genetic differentiation with F_{ST} values ranging between 0.006–0.892 was found. Five distinct genetic groups of the red-spotted tokay populations could be classified. A group of populations from northern Thailand showed the highest genetic differentiation from the other groups. Moreover, there was a substantial genetic subdivision depending on the genetic groups with $F_{CT} = 0.664$ and $F_{SC} = 0.185$. This genetic structure is related to geographical distribution and distance between populations, $R^2 = 0.5614$, $p < 0.001$. Our findings of pronounced genetic structuring and the concomitant conservation genetic consequences if further population loss occurs mean that management actions should therefore focus on the conservation of all of the main sites where tokay geckos still occur.

Introduction

Animal trafficking for traditional medicines or for the pet trade is a major issue in conservation biology (Baker et al 2013). The removal of breeding adults from wild populations of endangered species can potentially lead to extinction in the wild, while for common species it may mean the reduction of populations to endangered status (Schipper et al 2008). This is particularly true for Southeast Asia where most trafficking occurs (Nijman 2010; Caillabet 2013), and where reptile species, in particular, are endangered due their role in traditional Chinese medicine (Nijman et al 2012). One method of monitoring and potentially controlling such trafficking is to use genetic markers to define the local origin of populations and to convict offenders (Caniglia et al 2010; Barbanera et al 2002; White et al 2012). In order to do this, genetic variation and the spatial distribution of the various genetic groups must be known.

The tokay gecko (*Gekko gecko*) is the second largest gecko species known. There are two subspecies currently recognized, namely *G. g. gecko* (Linneaeus, 1758), which is found in tropical regions from

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northeast India and Bangladesh to southern China, and throughout Southeast Asia, i.e. Malaysia, Indonesia, the Philippines, Myanmar, Vietnam, Lao People's Democratic Republic (PDR), Cambodia, and Thailand. It has also been introduced to parts of the USA, West Indies, and Madagascar (Das 2010). The other subspecies is *G. g. azhari* Mertens, 1955, found only in Bangladesh.

The tokay gecko is a traditional Chinese medicine used to relieve various illness, such as diabetes, asthma, skin disease, and cancer (Li et al 2004; Bauer 2009). Its population in China has dramatically declined due to excessive hunting and habitat destruction (e.g. increasing urbanization). Thus, it has become an endangered species in China (Li et al 1996). Recently, advertisements were published, especially in Malaysia, Indonesia, and the Philippines, indicating that the consumption of tokay gecko tongues and internal organs are a cure for human immunodeficiency virus and cancer (Caillabet 2011; Caillabet 2013). This has led to these geckos being massively hunted in Southeast Asia for trade, especially in Thailand. From Thailand alone, around 2–5 million tokay geckos are exported to China, Taiwan, Malaysia, and USA each year (Laoong and Sribundit 2006). Tokay geckos are hunted at night when they are active, from March to October. They are sold to a middleman and usually transported to the Nawa district, Sakon Nakhon province, Thailand for processing. Here they are killed, disemboweled, stretched across bamboo sticks, and dried before export, mostly to China (Thongsa-Ard and Thongsa-Ard 2003; Caillabet 2011). To make long-term conservation decisions, we need information on population structure and connectivity (Moritz 1994). Dispersal ability, geographic variation, level of genetic differentiation, and adaptation to local conditions are key aspects of a species' ecology (Frankham et al 2002; Hartl and Clark 2007).

Recently, two different morphs of tokay geckos were recognized. The black-spotted tokay gecko is distributed across Guangxi and Yunnan Provinces, southern China and the red-spotted tokay gecko is mainly found through Southeast Asia (Qin et al 2012). There are numerous reports on the biology, and morphological and genetic differences between these two different morphs, e.g. body color and spots (Zhang et al 2006), advertisement calls (Yu et al 2012), karyotype (Qin et al 2012), random amplified polymorphic DNA patterns (Qin et al 2005), nuclear DNA (Wang et al 2013), mitochondrial DNA (Liu et al 2000; Zhang et al 2006; Qin et al 2012; Wang et al 2013), and microsatellite DNA (Peng et al 2010). This suggests that *G. gecko* is subdivided into two subspecies or species (Qin et al 2012). The intraspecific variation of black-spotted geckos in southern China has been intensively investigated (Liu et al 2000; Qin et al 2005; Peng et al 2010), whereas the genetic variation within the red-spotted gecko population in Southeast Asia is largely unknown. Moreover, previous studies on the latter morph were performed on few samples, which were from scattered localities and only represent partial geographical populations from Southeast Asia. Thus, unrecognized taxa or genetic groups among red-spotted tokay populations may have been missed (Wang et al 2013).

There are many potential molecular markers/techniques for investigating the genetic variation of tokay geckos. One of these techniques is multilocus enzyme electrophoresis. This has been successfully used to explore the genetic variation, genetic differentiation, and population genetics of the sympatric geckos, i.e. *Gekko tawaensis* and *Gekko japonicus* in Japan (Toda et al 2003; Toda et al 2006) and the Australian *Oedura marmorata* complex (Oliver et al 2014) among others. In order to obtain a better understanding of the systematic and taxonomic status of red-spotted *G. gecko* in Southeast Asia, the intrapopulation and interpopulation genetic variation and genetic structure of populations collected from different localities in Thailand, Lao People's Democratic Republic, and Cambodia were determined with multilocus enzyme electrophoresis.

Materials and methods

Sample collection

Live tokay geckos were caught by villagers from different localities in Thailand and neighboring countries and transported to the Nawa District, Sakon Nakhon province for processing and trading. A total of 125 liver samples of red-spotted tokay geckos from 16 different geographical localities were obtained (Table 1, Figure 1). The livers were removed after dissection then frozen in dry ice for transfer to the molecular laboratory at Walai Rukhavej Botanical Research Institute, Mahasarakham University where they were stored at −80°C until electrophoresis was carried out. The livers of two other species of house geckos, *Hemidactylus frenatus* and *H. platyurus* were used as an out-group.

Multilocus enzyme electrophoresis

Enzyme homogenates were individually prepared from a small piece of liver and ground manually with an equal volume of lysing solution (100 mL distilled water, 100 μL β-mercaptoethanol, 10 mg nicotinamide adenine dinucleotide phosphate) using a glass rod. These were then centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatants were stored in capillary tubes as 5 μL aliquots at −20°C until used. Multilocus enzyme electrophoresis was performed by using cellulose acetate (Cellogel, Milan) as the support medium. Each gel was stained histochemically for a specific enzyme (Richardson et al 1986). The 28 enzymes screened and their Enzyme Commission (E.C.) numbers were: aconitate dehydrogenase (E.C. 4.2.1.3), adenylate kinase (E.C. 2.7.4.3), aldolase (E.C. 4.1.2.13), enolase (E.C. 4.2.1.11), fructose-1,6-diphosphatase (E.C. 3.1.3.11), fumarate hydratase (E.C. 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12), glutamate dehydrogenase (E.C. 1.4.1.3), glucose dehydrogenase (E.C. 1.1.1.47), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), aspartate amino transferase (E.C. 2.6.1.1), glucose-phosphate isomerase (E.C. 5.3.1.9), alanine amino transferase (E.C. 2.6.1.2), β-hydroxybutyrate dehydrogenase (E.C. 1.1.1.30), hexokinase (E.C. 2.7.1.1), isocitrate dehydrogenase (E.C. 1.1.1.42), lactate dehydrogenase (E.C. 1.1.1.27), malate dehydrogenase (E.C. 1.1.1.37), malic enzyme (E.C. 1.1.1.40), mannose-phosphate isomerase (E.C. 5.3.1.8), purine nucleotide phosphorylase (E.C. 2.4.2.1), peptidase phenyalanine-proline (E.C. 3.4.13), phosphoglycerate kinase (E.C. 2.7.2.3), phosphoglucomutase (E.C. 2.7.5.1), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44), pyruvate kinase (E.C. 2.7.1.40), superoxide dismutase (1.15.1.1), and L-iditol dehydrogenase (E.C. 1.1.1.14).

Table 1. Geographical areas of sample collection.

Code	*N*	District	Province	Region	Country
NR	9	Sikhio	Nakhon Ratchasima	Northeast	Thailand
CP	6	Thep Sathit	Chaiyaphum	Northeast	Thailand
KK	10	Kranuan	Khon Kaen	Northeast	Thailand
KS	11	Kham Muang	Kalasin	Northeast	Thailand
SK	5	Sawangdandin	Sakon Nakhon	Northeast	Thailand
UD	5	Ban Dung	Udon Thani	Northeast	Thailand
MH	10	Don Tan	Mukdahan	Northeast	Thailand
UB	8	Khemarat	Ubon Ratchathani	Northeast	Thailand
SR	5	Sikhoraphum	Surin	Northeast	Thailand
LP	10	Mueang	Lampang	North	Thailand
LN	5	Li	Lamphun	North	Thailand
CR	5	Wiang Pa Pao	Chiang Rai	North	Thailand
PY	12	Mueang	Phayao	North	Thailand
VT	7	Kampang Nakhon	Vientiane	North	Lao PDR
SV	7	Songkhone	Savannakhet	Central	Lao PDR
CD	10	Siem Reap	Siem Reap	North	Cambodia

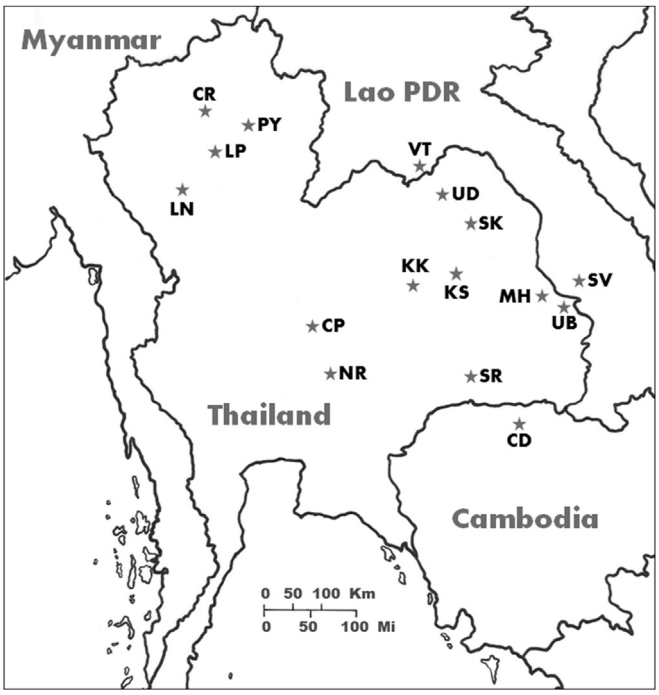

Figure 1. Map of sampling localities of red-spotted tokay gecko. CD = Siem Reap; CP = Chaiyaphum; CR = Chiang Rai; KK = Khon Kaen; KS = Kalasin; LN = Lamphun; LP = Lampang; MH = Mukdahan; NR = Nakhon Ratchasima; PY = Phayao; SK = Sakon Nakhon; SR = Surin; SV = Savannakhet; UB = Ubon Ratchathani; UD = Udon Thani; VT = Vientiane.

Allele scoring and data analyses

For each locus, the electrophoretic banding pattern of each sample was interpreted allozymically, that is, the allozyme with the least electrophoretic mobility from the cathode was designated as allele *a*. A phenogram was constructed using the Unweighted Pair–Group Method with Arithmetic averages (Sneath and Sokal 1978) analysis of pair wise comparisons of the proportion of loci that showed fixed allelic differences between red-spotted tokay geckos from different localities. Allele frequency and genetic differentiation (F_{ST} value) were calculated by GenePop on the web program. The genetic structure was generated based on analysis of molecular variance using program Arlequin 3.5 (Excoffier and Lischer 2010). The correlation between genetic and geographic distances was calculated using Isolation by Distance web service version 3.23 (Jensen et al 2005).

Results

Of the 28 enzyme systems examined in the current study, 25 enzymes encoding presumptive 29 loci gave sufficient staining for reliable interpretation. Allelic variability within and between populations was observed at seven loci, i.e. *G6pd*, *Ldh*, *Mpi*, *PepD*, *6Pgd*, *Pgm-1*, and *Sod* (Table 2). Genetic differentiation was observed between the 16 populations with F_{ST} values ranging between 0.006 and 0.892 (Table 3). All pairwise F_{ST} showed significant differences ($p < 0.01$) except for Chaiyaphum versus Nakhon Ratchasima, Kalasin versus Khon Kaen, Udon Thani versus Sakon Nakhon, Vientiane versus Savannakhet, Ubon Ratchathani versus Mukdahan and between the populations from north Thailand, namely Lampang, Lamphun, Chiang Rai, and Phayao (Table 3).

Five groups (I–V) were observed in a phenogram generated from the allelic profile of all 29 loci examined (Figure 2). Group I contained the four populations, i.e. Surin, Chaiyaphum, and Nakhon Ratchasima from northeast Thailand and a population from Cambodia (Siem Reap). Group II comprised the populations of Mukdahan and Ubon Ratchathani from northeast Thailand. These groups are closely aligned at 3% fixed differences (Figure 2). The other four populations from northeast Thailand, namely Khon Kaen, Kalasin, Sakon Nakhon, and Udon Thani, were aligned as group III. Group IV contained the two populations of Vientiane and

Table 2. Allele frequencies of seven polymorphic loci of 16 geographical samples of red-spotted tokay gecko, including house geckos, *Hemidactylus platyurus* (Hp), and *H. frenatus* (Hf).

Locus	*allele*	Allele frequency of red-spotted *G. gecko* populations																*Hp*	*Hf*
		NR	CP	KK	KS	SK	UD	MH	UB	SR	LP	LN	CR	PY	VT	SV	CD		
G6pd	*a*																	1.00	1.00
	b			1.00	1.00	1.00	1.00				1.00	1.00	1.00	1.00	0.29				
	c	1.00	1.00							0.80							0.65		
	d							1.00	1.00	0.20					0.71	1.00	0.35		
Ldh	*a*							0.75	0.75	0.80					0.86	0.79	0.10		
	b	0.67	0.75	0.70	0.86	0.80	0.70	0.25	0.25	0.20	0.70	0.80	0.90	0.75		0.21	0.90		
	c	0.33	0.25	0.30	0.14	0.20	0.30				0.30	0.20	0.10	0.25	0.14				
	d																	1.00	1.00
Mpi	*a*					0.70	0.70	0.85	0.87					0.04	0.21	0.29		1.00	
	b	1.00	1.00	1.00	1.00	0.30	0.30	0.15	0.23	1.00	1.00	1.00	1.00	0.96	0.79	0.71	1.00		1.00
PepD	*a*	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50			0.50	1.00	1.00
	b	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50					0.50	0.50	0.50		
	c										0.50	0.50	0.50	0.50	0.50	0.50			
6Pgd	*a*										0.20	0.10	0.20	0.17					
	b			1.00	1.00	1.00	1.00	0.90	1.00						1.00	1.00			
	c	1.00	1.00							1.00	0.80	0.90	0.80	0.83			1.00	1.00	
	d							0.10											1.00
Pgm-1	*a*																	1.00	1.00
	b										1.00	0.90	1.00	1.00					
	c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.10					0.10		
	d														1.00	1.00	0.90		
Sod	*a*										1.00	1.00	1.00	1.00					
	b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					1.00	1.00	1.00		
	c																	1.00	1.00

CD = Siem Reap; CP = Chaiyaphum; CR = Chiang Rai; KK = Khon Kaen; KS = Kalasin; LN = Lamphun; LP = Lampang; MH = Mukdahan; NR = Nakhon Ratchasima; PY = Phayao; SK = Sakon Nakhon; SR = Surin; SV = Savannakhet; UB = Ubon Ratchathani; UD = Udon Thani; VT = Vientiane.

Table 3. Pairwise comparison of F_{ST} value between 16 populations of red-spotted tokay gecko.

Code	NR	CP	KK	KS	SK	UD	MH	UB	SR	LP	LN	CR	PY	VT	SV	CD
NR	–															
CP	0.036	–														
KK	**0.671**	**0.674**	–													
KS	**0.705**	**0.711**	0.006	–												
SK	**0.690**	**0.683**	0.288*	**0.333**	–											
UD	**0.679**	**0.668**	0.270†	**0.329**	0.096	–										
MH	**0.755**	**0.740**	**0.832**	**0.840**	**0.779**	**0.780**	–									
UB	**0.734**	**0.720**	**0.868**	**0.878**	**0.828**	**0.826**	0.037	–								
SR	**0.620**	**0.585**	**0.835**	**0.848**	**0.807**	**0.804**	**0.743**	**0.604**	–							
LP	**0.684**	**0.665**	**0.763**	**0.780**	**0.739**	**0.734**	**0.848**	**0.817**	**0.678**	–						
LN	**0.687**	**0.662**	**0.765**	**0.788**	**0.725**	**0.718**	**0.846**	**0.819**	**0.661**	0.048	–					
CR	**0.751**	**0.737**	**0.819**	**0.839**	**0.790**	**0.783**	**0.871**	**0.848**	**0.705**	0.012	0.048	–				
PY	**0.661**	**0.642**	**0.741**	**0.756**	**0.717**	**0.713**	**0.835**	**0.801**	**0.661**	0.036	0.054	0.010	–			
VT	**0.660**	**0.617**	**0.706**	**0.715**	**0.637**	**0.641**	**0.526**	**0.606**	**0.642**	**0.793**	**0.758**	**0.790**	**0.787**	–		
SV	**0.824**	**0.815**	**0.867**	**0.877**	**0.840**	**0.838**	**0.623**	**0.727**	**0.804**	**0.869**	**0.867**	**0.892**	**0.859**	0.017	–	
CD	0.191*	0.138*	**0.717**	**0.733**	**0.710**	**0.707**	**0.629**	**0.553**	**0.432**	**0.705**	**0.697**	**0.746**	**0.685**	**0.601**	**0.753**	–

Bold $p < 0.0001$.
CD = Siem Reap; CP = Chaiyaphum; CR = Chiang Rai; KK = Khon Kaen; KS = Kalasin; LN = Lamphun; LP = Lampang; MH = Mukdahan; NR = Nakhon Ratchasima; PY = Phayao; SK = Sakon Nakhon; SR = Surin; SV = Savannakhet; UB = Ubon Ratchathani; UD = Udon Thani; VT = Vientiane.
* $p < 0.01$.
† $p < 0.001$.

Savannakhet from Lao People's Democratic Republic. Group III and IV are closely aligned at 5% fixed differences. A cluster of group I and II was aligned with the cluster of group III and IV at 5.5% fixed differences. Group V contained the four populations Lamphun, Lampang, Phayao, and Chiang Rai from north Thailand. It was clustered and showed the highest genetic differentiation to other groups at 9% fixed differences (Figure 2). In addition, the red-spotted tokay was genetically distinct from the other two species of house geckos tested at 45% fixed differences.

Substantial genetic subdivision and low gene flow existed among the five genetically distinct groups, with a highly significant F_{ST} value (Table 3). Significances of $p < 0.001$ for $F_{CT} = 0.664$, $F_{SC} = 0.185$, and $F_{IT} = 0.668$ were observed among groups, among populations within groups, and within individuals, respectively (Table 4). Our results also showed that there was deviation from Hardy–Weinberg expectations, with a highly significant heterozygote deficiency in all polymorphic loci as indicated by the significant, positive F_{IS} value (Table 5). All genetic groups showed a significant deficiency of heterozygous at *Ldh*, group IV at *G6pd*, groups II, III, and IV at *Mpi*, and groups I and II at *6Pgd* (Table 5).

A strong correlation between genetic difference (F_{ST}) and geographic distances among local populations was observed with $R^2 = 0.5614$, $p < 0.001$ (Figure 3). These results suggest that

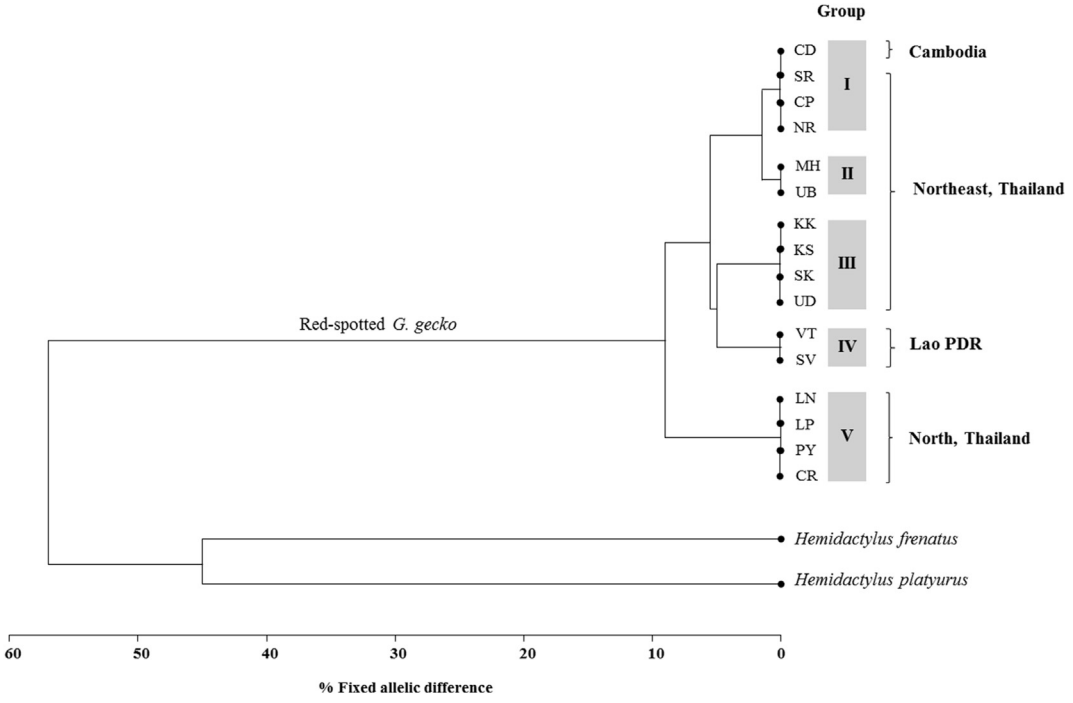

Figure 2. Phenogram based on the percentage of fixed differences among 16 populations of red-spotted tokay gecko using *Hemidactylus frenatus* and *H. platyurus* as out group. CD = Siem Reap; CP = Chaiyaphum; CR = Chiang Rai; KK = Khon Kaen; KS = Kalasin; LN = Lamphun; LP = Lampang; MH = Mukdahan; NR = Nakhon Ratchasima; PY = Phayao; SK = Sakon Nakhon; SR = Surin; SV = Savannakhet; UB = Ubon Ratchathani; UD = Udon Thani; VT = Vientiane.

Table 4. Analysis of molecular variance of 16 populations of red-spotted tokay gecko classified into five genetic groups (Figure 2).

Source of variation	d.f.	Sum of squares	Variance components	% variation	Fixation indices
Among groups	4	295.59	1.44	66.38	$F_{CT} = 0.664$*
Among populations within group	11	27.51	0.13	6.21	$F_{SC} = 0.185$*
Among individuals within populations	109	51.17	0.12	5.77	$F_{ST} = 0.211$
Within individuals	125	90.00	0.72	21.64	$F_{IT} = 0.668$*

Level of significance based on 1000 permutations is indicated by * = $p < 0.001$.

geographical distance is an important factor limiting gene flow between red-spotted tokay populations.

Discussion

Our study provides the first data on the genetic structure of populations of red-spotted tokay from different geographical areas in Thailand, Lao PDR and Cambodia. As such, it represents the first study providing information that can be used for forensic control of wildlife trade for this species.

We found that substructuring occurred between the 16 populations of the red-spotted tokay examined according to their geographical origin in Thailand, Lao PDR and Cambodia, similar to that described for another morph, black-spotted tokay in southern China (Zhang et al 2006). The genetic differences found for red-spotted tokay populations from different localities in the current study are probably due to a lack or low level of gene flow, especially between spatially distant populations, or populations separated by natural barriers. For instance, the populations from northern Thailand are blocked and separated from populations on the Khorat Plateau of northeastern Thailand by the Dong Phaya Yen mountain range. This range has also been implicated as a barrier to gene flow between rice-field frog (*Hoplobatrachus rugulosus*) populations (Pansook et al 2012). The northeastern of Thailand is separated from Lao PDR by the Mekong River, which probably acts as a natural barrier to gene flow as suggested by Tantrawatpan et al (2011) for the cricket *Brachytrupes portentosus*, as well as for various amphibian species (Geissler et al 2015). This hypothesis is supported by the lack of differentiation between the Thai population nearest Cambodia (Sikhoraphum) and the Cambodian (Siem Reap) population, which are separated only a very narrow section of the Dângrêk Mountains. Nevertheless, the populations in the northeast region showed significant genetic differentiation between groups I, II, and III, which may be separated by fragmented forests or wetlands.

Our current investigation found that intraspecific variation within the red-spotted tokay geckos in Thailand and Lao PDR was

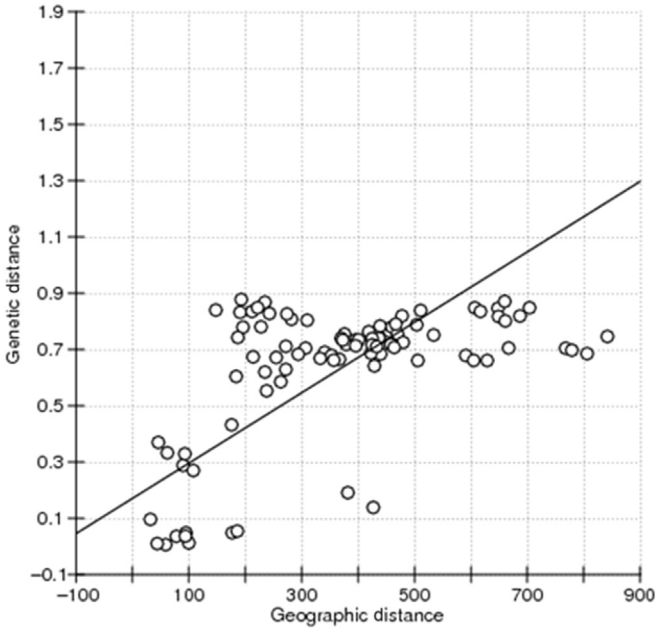

Figure 3. Correlation graph plotted between genetic distance (F_{ST} value) against geographic distance (km) for 125 individual samples of red-spotted tokay from 16 populations, $R^2 = 0.5614$; $p < 0.001$.

quite low, as indicated by significant heterozygote deficiency (positive F_{IS} value) in all groups of populations at almost all polymorphic loci. However, the genetic differentiation among populations strongly indicates that the red-spotted tokay populations in Southeast Asia, and especially in Thailand are subdivided based on their geographical origin. The heterozygote deficiency compared to HWE strongly suggests that migration between populations was very low. These findings are probably affected by a decrease in effective population sizes and reduced gene flow between populations.

Nevertheless, the marked genetic differences among populations that still remain, and the profusion of private alleles, highlight that any further population loss, even of the small-sized populations, will lead to significant losses of genetic diversity. The loss of genetic diversity will be particularly evident if any of the main five genetic groups, which likely represent different evolutionary lineages, goes extinct. Management actions should therefore focus on the conservation of all of the main sites where tokay geckos still occur.

Our findings of pronounced genetic structuring, and the concomitant conservation genetic consequences if further population loss occurs, may therefore apply to a wide range of endangered rare endemic reptile species in Southeast Asia. In the future, DNA studies should be used to confirm the spatial variation found

Table 5. The expected (H_e) and observed (H_o) heterozygosity of four polymorphic loci of red-spotted tokay gecko populations.

Populations*	N†	*G6pd*			*Ldh*			*Mpi*			*6Pgd*		
		H_e	H_o	F_{IS}	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}	H_e	H_o	F_{IS}
Group I	30	0.259	0.167	0.361	0.491	0.300	0.393‡	–	–	–	0.235	0.067	0.719§
Group II	18	–	–	–	0.386	0.167	0.575§	0.246	0.167	0.329§	0.514	0.111	0.789§
Group III	31	–	–	–	0.357	0.258	0.277§	0.355	0.064	0.821§	–	–	–
Group IV	14	0.218	0.143	0.447§	0.320	0.214	0.339§	0.388	0.214	0.458§	–	–	–
Group V	32	–	–	–	0.364	0.156	0.575§	0.031	0.031	0.000	0.222	0.125	0.441

– monomorphic locus: no test done.
* Population defined by genetic group in Figure 2.
† Sample size.
‡ $P < 0.01$.
§ $P < 0.001$.

and to construct markers for forensic use. In addition, morphological and biological data should be collected to determine the taxonomic status of the various groups: whether they are races, subspecies or whether the tokay gecko represents a species complex.

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