Insular shifts and trade-offs in life-history traits in pond frogs in the Zhoushan Archipelago, China

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Abstract

Island and mainland populations of animal species often differ strikingly in life-history traits such as clutch size, egg size, total reproductive effort and body size. However, despite widespread recognition of insular shifts in these life-history traits in birds, mammals and reptiles, there have been no reports of such life-history shifts in amphibians. Furthermore, most studies have focused on one specific life-history trait without explicit consideration of coordinated evolution among these intimately linked life-history traits, and thus the relationships among these traits are poorly studied. Here we provide the first evidence of insular shifts and trade-offs in a coordinated suite of life-history traits for an amphibian species, the pond frog *Rana nigromaculata*. Life-history data were collected from eight islands in the Zhoushan Archipelago and neighboring mainland China. We found consistent, significant shifts in all life-history traits between mainland and island populations. Island populations had smaller clutch sizes, larger egg sizes, larger female body size and invested less in total reproductive effort than mainland populations. Significant negative relationships were found between egg size and clutch size and between egg size and total reproductive effort among frog populations after controlling for the effects of body size. Therefore, decreased reproductive effort and clutch size, larger egg size and body size in pond frogs on islands were selected through trade-offs as an overall life-history strategy. Our findings contribute to the formation of a broad, repeatable ecological generality for insular shifts in life-history traits across a range of terrestrial vertebrate taxa.

Introduction

Island and mainland populations of animal species often differ strikingly in their life-history traits. There is strong evidence that both the direction and the magnitude of island–mainland differences in life-history evolution are predictable. Compared with mainland populations, insular populations are generally typified by a smaller clutch size (Ricklefs, 1980; Crowell & Rothstein, 1981; Blondel, 1985; Fitch, 1985), a larger offspring size (Stamps & Buechner, 1985; Fons et al., 1997; Wiggins et al., 1998) and decreased reproductive effort (i.e. resources allocated to reproduction, the product of clutch size and egg size; Andrews, 1979; Fons et al., 1997; Caley, Schwarzkopf & Shine, 2001). Despite widespread recognition of insular shifts in these life-history traits in birds, mammals and reptiles, there have been no reports of such life-history shifts in amphibians. Furthermore, most studies have focused on one specific life-history trait without explicit consideration of coordinated evolution among these intimately linked life-history traits. Thus,

there is a paucity of information on the simultaneous evolution of reproductive traits in animal populations inhabiting islands.

Two lineages of life-history theory have been developed to predict the evolution of reproductive effort and offspring size (Smith & Fretwell, 1974; Winkler & Wallin, 1987). Both theory lineages predict that the simultaneous evolution of offspring size and number is constrained by a trade-off between these two traits. An assumption of one theory is that allocations to total reproductive effort and offspring size involve a two-step process: the decision about optimal allocation of resources to reproductive effort is made first, followed by the decision about the optimal partitioning of that investment into a few large versus many small offspring (Smith & Fretwell, 1974; Roff, 1992; Stearns, 1992). Under this model, offspring size and total reproductive effort are not directly correlated. An alternative model predicts a direct, negative relationship between evolutionary shifts in offspring size and reproductive effort (Winkler & Wallin, 1987). A negative relationship between reproductive effort

and offspring size could arise through independent selection on each of these traits, or if genes for egg size are linked with genes for total reproductive effort, to produce a secondary (i.e. non-direct) correlation between these two traits (Caley *et al.*, 2001). While there is relatively strong support for a trade-off between offspring size and number in animal populations (Fox, 1997; Fischer *et al.*, 2006; Warne & Charnov, 2008), the evidence for a link between reproductive effort and offspring size has been controversial (Schwarzkopf, Blows & Caley, 1999; Caley *et al.*, 2001; Czesak & Fox, 2003; Fischer *et al.*, 2006; Karl, Lorenz & Fischer, 2007).

The pond frog *Rana nigromaculata* on land-bridge archipelagos is an ideal species to test life-history theories concerned with reproductive strategies and trade-offs across macroecological spatial scales. First, because sea water is harmful to amphibians (Nussbaum, 1984), frogs cannot disperse among islands and the mainland, and the current distribution of amphibians on islands is due to selective extinction since isolation (Yiming, Niemelä & Dianmo, 1998). This eliminates the effects of immigration and dispersal of individuals among islands and the mainland on the evolution of frog life-history strategies. Thus, each island is a closed system and the frogs on each island are often hypothesized to have evolved independently (Wu, Li & Murray, 2006). Second, female pond frogs produce a single clutch each year and there is no parental care for eggs. This largely controls for the potential confounding effects of multiple breeding events in the same year on reproductive effort, egg size and clutch size. Therefore, clutch mass directly reflects the reproductive effort of parents on offspring for a given year. Finally, the pond frog is one of the most widespread and abundant amphibians in the study area that allows measurements to be made using large sample sizes (Huang, 1990).

In this study, we investigated variations in total reproductive effort, egg size, clutch size and female body size in pond frog populations on eight islands in the Zhoushan land-bridge Archipelago (East China Sea) and neighboring mainland China (Zhejiang province). We addressed the following questions: (1) Do island and mainland populations of pond frogs differ in clutch size, egg size, total reproductive effort and body size? (2) Is there a trade-off between egg size and clutch size among island populations? (3) Is there a negative relationship between egg size and reproductive effort?

Materials and methods

Study sites and species

The study was carried out in the Zhoushan Archipelago (29°31′–31°04′N, 121°30′–123°25′E) and on the neighboring mainland (29°49′–29°54′N, 121°55′–122°04′E) in the northeast part of Zhejiang province, China (Fig. 1). The Zhoushan Archipelago is the largest archipelago in China. It contains 1339 islands, ranging in size from small rocks to the largest island (Zhoushan), with an area of 468.7 km². The total area of the archipelago is about 1371 km². The archipelago was originally a part of the neighboring
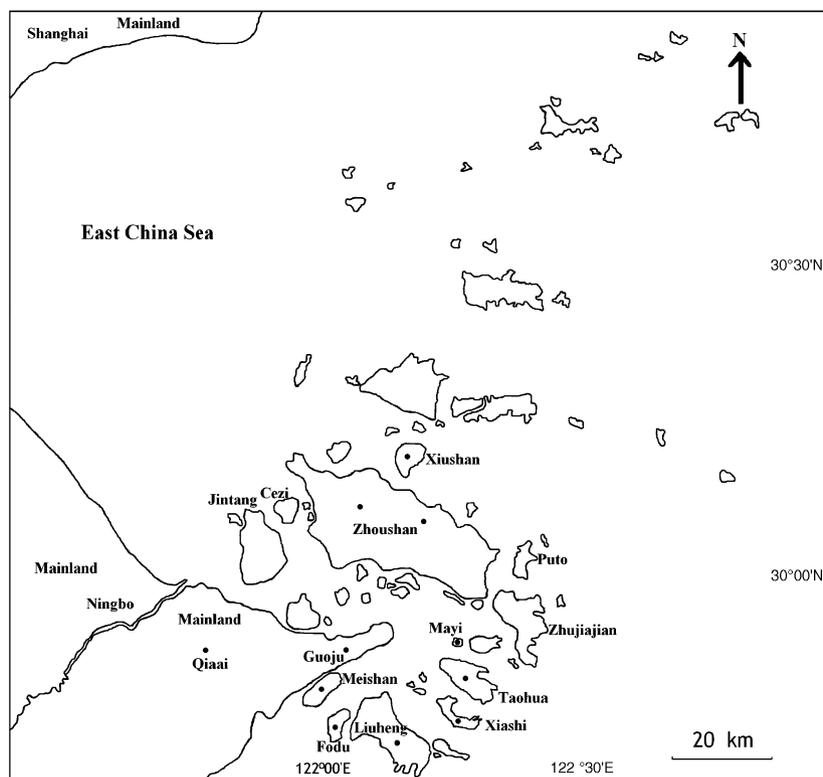
Figure 1 Locations of sampling sites (filled circles) in the Zhoushan Archipelago and on neighboring mainland China.

mainland, but separated from it about 7000–9000 years ago due to rising sea levels during the late Pleistocene (Wang & Wang, 1980; Zhou, 1987). As a land-bridge archipelago, the topography, climate, vegetation and fauna on the islands are very similar to the mainland (Zhou, 1987; Huang, 1990; Zhuge, 1990). Natural vegetation is dominated by subtropical evergreen broadleaf forest. The climate is typical of the subtropical ocean monsoon zone and is highly seasonal, with a mean temperature ranging from $5.7\,°C$ in January to $26.7\,°C$ in July. Annual rainfall is about 1200–$1400\,mm$. Species richness on the islands is poorer than on the mainland, with 10 amphibian species found in the Zhoushan Archipelago compared with 17 species on the mainland (Huang, 1990; Yiming *et al.*, 1998). Furthermore, only 24 islands support amphibian populations (Yuan, 1965; Yiming *et al.*, 1998).

Pond frogs are widely distributed in most provinces of China (Huang, 1990; Fei, 1999) and occur on most islands above $1\,km^2$ in the Zhoushan Archipelago (Yiming *et al.*, 1998). On the islands and nearby mainland, pond frogs generally emerge from hibernation in March and breed between late March and early July. The frogs are commonly found in rice fields, ditches, rivers, ponds, reservoirs and surrounding areas. Eggs are found attached to submerged vegetation in the shallow water of these habitats. Clutch size ranges from 780 to 5514 eggs. Eggs hatch into tadpoles within 3 or 4 days. The tadpoles then transform into juveniles in about 60 days. Hibernation usually begins in early November (Huang, 1990).

Frog sampling methodology

We selected eight islands (Zhoushan, Liuheng, Taohua, Xiushan, Meishan, Xiashi, Fodu and Mayi; Fig. 1) and two locations on the mainland for our study. The islands sampled are all close to each other and the mainland, thus reducing the effects of differences in climate on frog reproduction. We sampled at one location on each of the islands, but because Zhoushan is much larger in area than the other islands, we sampled two locations on Zhoushan (Fig. 1). No frog hunting takes place at any of our sampled sites.

The study was conducted from April to June during the frog breeding season between 2004 and 2005. At each location, we collected data on clutch size, egg size and body size. Data were collected on islands once and at the mainland locations twice (Table 1). We began data collection in the field on Guoju and Qiaai and then a random number table was used to determine the order of sampling of the studied islands. After we finished sampling on the islands, we re-sampled Guoju and Qiaai to minimize any potential effects of sampling time (Wu *et al.*, 2006). We carried out surveys at each location generally for 3–4 consecutive days. All study islands, except Mayi and Meishan, have been invaded by American bullfrogs *Rana catesbeiana* (Yiming, Zhengjun & Duncan, 2006). To avoid the potential effects of bullfrogs on pond frogs, all data were collected in areas and habitats where bullfrogs had not yet invaded and all study sites were at least 2 km away from invaded areas.

Table 1 Sampling date, island area and distance of each island to the mainland for the study sites in the Zhoushan Archipelago and neighboring mainland

Site	Sampling date		Area (km²)	Distance to mainland (km)
	2004	2005		
Mainland	May 14/June 7	April 15/May 27		
Zhoushan	June 4	May 23	468.7	9.0
Liuheng	May 22	April 24	92.8	7.0
Taohua	May 17	May 12	41.0	8.8
Xiushan	June 15	May 20	22.3	26.8
Meishan	June 19	April 29	21.9	0.5
Xiashi	May 20	April 20	16.7	13.2
Fodu	June 2	April 26	7.0	7.0
Mayi	May 28	May 6	2.2	11.5

Egg collection and measurement

During the study period, we searched for pond frog clutches thoroughly in the accessible areas of potential breeding habitats at each study site between 08:30 and 16:00 h every day. Once found, each recently laid clutch was retrieved as a whole, placed into a plastic basin (*c.* 20 cm in diameter), then carefully placed in separate plastic bags with water and taken to the laboratory for further analysis. Indoors, each clutch was spread out in a white tray and counted with the aid of an arithmometer. Using vernier calipers, we measured the yolk diameter to the nearest 0.02 mm of six eggs from each clutch to estimate the average egg size for each clutch. We obtained a representative selection by measuring eggs from the center, edges, top and bottom of the clutch (Lips, 2001). Only eggs before the Gosner 10 stage were measured, because after this stage the embryo assumes an oval shape and increases in size (Gosner, 1960). Clutches that had developed after Gosner 10 were excluded from further examination.

Frog collection and measurement

We captured pond frogs by a dip-net or by hand along line transects where frog clutches were gathered. Sampling took place at night between 19:00 and 21:30 h with a 12 V DC lamp. All captured frogs were placed in plastic bags and taken indoors for further analysis. Indoors, frogs were measured and sexed. We measured the snout–vent length (SVL, with vernier calipers to the nearest 0.02 mm) and body mass (with a 100 g balance to the nearest 0.1 g) of all individuals. The gender of sexually mature frogs was determined by the presence or absence of secondary sexual characters, such as nuptial pads and linea masculina (Huang, 1990; Fei, 1999). Owing to independent evolution of frog body size on each island and the mainland, individuals with SVL less than the minimum confirmed male SVL of pond frogs on the mainland and each island were considered juveniles there, and frogs without male characteristics were considered females (Wu *et al.*, 2006). Frogs were then released the next morning at the sites where they were captured.

Island characteristics

Data on island areas were obtained from Chen (1989). The distances of each island to the mainland were measured on a map at a scale of 1:400 000.

Statistical analyses

Our analyses deal entirely with female frogs because they have greater effects on reproductive traits than males (Stearns, 1992). We used SVL, rather than body mass, to represent the body size of female frogs mainly for two reasons. First, egg masses make up a large proportion of a female's body weight before she lay eggs (Ryser, 1989), which would dramatically bias the weight measurement. Moreover, body mass varies considerably with the amount of urine in the bladder (up to 50% of body mass in fully hydrated individuals for some frog species) (Pough *et al.*, 1998). In contrast, SVL does not change with the gravid status or the amount of urine, and thus is a more appropriate measure than body mass.

A preliminary analysis showed that clutch size, egg size and body size did not differ significantly between the two mainland locations, or between the two locations on Zhoushan. Thus, we pooled the samples from the two mainland locations into one sample, and repeated this process for the two locations on Zhoushan. In addition, there were no differences in the clutch size, egg size or body size between the two sampled years for Zhoushan, Liuheng, Taohua, Xiashi, Fudo, Mayi and the mainland (the sample sizes of clutch sizes on Meishan and Xiushan were too small to perform comparative analyses between 2 years). Thus, we pooled the samples from 2 years into one sample for the mainland population and for each island population. Following Caley *et al.* (2001), we calculated the total reproductive effort as the product of clutch size and egg size. We calculated the mean clutch size, egg size, total reproductive effort and SVL for each island and the mainland for use in the analyses described below. Because the relationships between clutch size, egg size, total reproductive effort and body size are frequently allometric (Roff, 1992),

these variables were ln-transformed. Island areas were also ln-transformed to meet the requirements of parametric tests. We used simple linear regressions to examine the relationships between life-history traits and island area and isolation.

We used *t*-tests to examine differences in clutch size, egg size, total reproductive effort and body size between the mainland and each island population. Because clutch size, egg size and total reproductive effort are commonly correlated with body size (Roff, 1992; Stearns, 1992), we needed to control for the effects of body size when examining the relationships among these life-history traits. One of the most primitive procedures to eliminate the size effect is residual analysis, but this technique is now frequently criticized for inherent flaws and biased parameter estimates (Darlington & Smulders, 2001; García-Berthou, 2001). In contrast, multiple regression provides unbiased parameter estimates while controlling for the effects of other variables in the model, which is practically appropriate when all the variables (both those of interest and the confounding variables) are continuous variables (Smith, 1999; Darlington & Smulders, 2001; Freckleton, 2002). Therefore, we used multiple regressions to control for maternal size effects when examining relationships between reproductive traits. For all analyses, two sets of regression analyses were performed. One set included both islands and mainland and the other set considered islands alone. All statistical analyses were performed using SPSS (SPSS Inc., 1998). All tests were two-tailed and statistical significance was set at $\alpha \leq 0.05$.

Results

There were significant shifts in all life-history traits of pond frogs between the mainland and all islands (Table 2). Island frog populations had significantly smaller clutch sizes and invested significantly less in total reproductive effort than mainland populations. There were significant shifts to larger egg size and female body size on all islands, except Zhoushan and Liuheng.

There were significant island size effects on all life-history traits of pond frogs. Clutch size (Fig. 2a) and total

Table 2 Differences in clutch size, egg size, total reproductive effort and female snout–vent length (SVL) of pond frogs between the mainland and eight islands of the Zhoushan Archipelago

Site	Clutch size (n) Mean ± SE (n)	P (t-test)	Egg size (mm) Mean ± SE (n)	P (t-test)	Total reproductive effort Mean ± SE (n)	P (t-test)	Female SVL (mm) Mean ± SE (n)	P (t-test)
Mainland	4878 ± 512 (12)		1.63 ± 0.02 (12)		7956.11 ± 848.94 (12)		73.45 ± 2.30 (24)	
Zhoushan	2610 ± 191 (12)	**0.001**	1.68 ± 0.01 (12)	0.064	4357.39 ± 294.61 (12)	**0.001**	71.64 ± 2.17 (33)	0.575
Liuheng	3002 ± 492 (11)	**0.016**	1.65 ± 0.02 (11)	0.518	4866.71 ± 731.28 (11)	**0.012**	71.10 ± 2.59 (21)	0.499
Taohua	2606 ± 186 (10)	**0.001**	1.79 ± 0.04 (10)	**0.001**	4626.43 ± 259.22 (10)	**0.002**	85.02 ± 2.38 (19)	**0.001**
Xiushan	2101 ± 370 (3)	**0.022**	1.85 ± 0.05 (3)	**0.001**	3844.19 ± 572.29 (3)	**0.037**	89.53 ± 2.41 (10)	**<0.001**
Meishan	2569 ± 335 (4)	**0.026**	1.80 ± 0.05 (4)	**0.001**	4595.23 ± 538.33 (4)	**0.046**	84.22 ± 2.31 (10)	**0.003**
Xiashi	2307 ± 346 (16)	**<0.001**	1.75 ± 0.02 (16)	**0.001**	4067.58 ± 628.02 (16)	**0.001**	86.60 ± 2.16 (19)	**<0.001**
Fodu	1680 ± 359 (10)	**<0.001**	1.82 ± 0.03 (10)	**<0.001**	2979.84 ± 592.51 (10)	**<0.001**	80.43 ± 2.48 (24)	**0.045**
Mayi	1560 ± 189 (12)	**<0.001**	1.95 ± 0.02 (12)	**<0.001**	3058.00 ± 378.53 (12)	**<0.001**	89.05 ± 2.00 (18)	**<0.001**

Total reproductive effort was calculated as the product of clutch size and egg size. n = sample size. P-values from t-tests are provided (significant results in bold type).
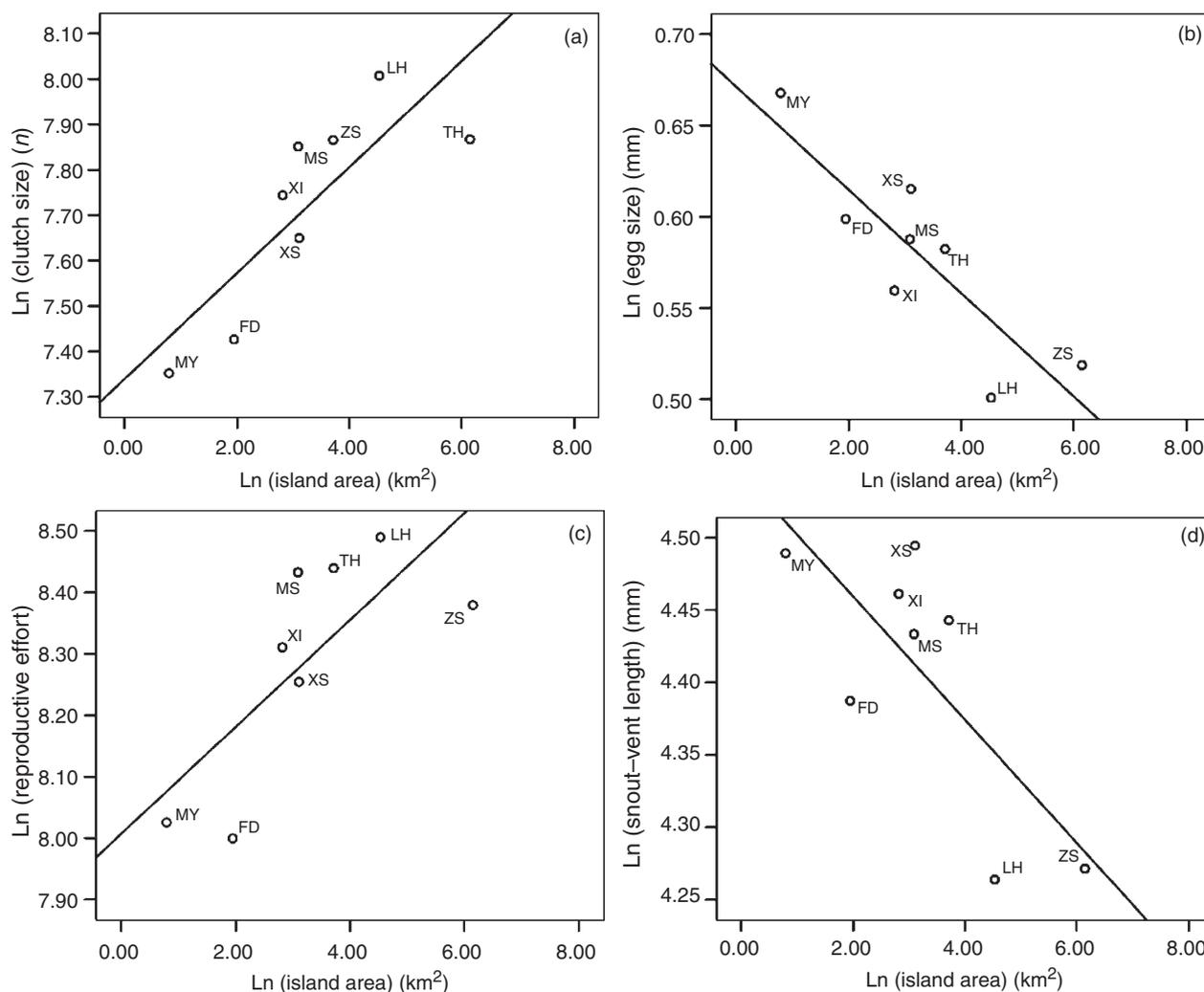
Figure 2 Relationships among island area and (a) clutch size ($r=0.820$, $n=8$, $P=0.013$), (b) egg size ($r=-0.858$, $n=8$, $P=0.006$), (c) reproductive effort ($r=0.748$, $n=8$, $P=0.033$) and (d) snout–vent length ($r=-0.751$, $n=8$, $P=0.032$) of pond frogs *Rana nigromaculata* in the Zhoushan Archipelago. ZS, Zhoushan; LH, Liuheng; TH, Taohua; XS, Xiushan; MS, Meishan; XI, Xiashi; FD, Fodu; MY, Mayi.

reproductive effort (Fig. 2c) increased significantly with island area, while egg size (Fig. 2b) and female body size (Fig. 2d) decreased significantly with island area. However, island isolation had no effects on life-history traits in pond frogs. Clutch size ($r=-0.262$, $n=8$, $P=0.530$), egg size ($r=0.293$, $n=8$, $P=0.482$), reproductive effort ($r=-0.229$, $n=8$, $P=0.585$) and female body size ($r=0.433$, $n=8$, $P=0.284$) were not significantly correlated with the distance of each island to the mainland.

There was a significant negative relationship between egg size and clutch size (Fig. 3a) and a marginally significant relationship between egg size and total reproductive effort (Fig. 3b) for islands alone. These relationships were strengthened when mainland and island populations were considered together (Fig. 3a and b). Also, after controlling for the effects of body size, we found a significant negative relationship between egg size and clutch size for islands alone and for the mainland and islands combined (Table 3).

There were also significant negative relationships between egg size and total reproductive effort for islands alone and for mainland and islands combined (Table 4).

Discussion

Our study provides the first evidence of coordinated shifts in life-history traits associated with reproductive strategies between island and mainland populations of an amphibian species. Island populations of pond frogs allocated less resources to reproduction, produced larger eggs and had smaller clutch sizes on nearly all islands compared with the mainland. These results are consistent with observations for other vertebrate taxa (mammals: Jewell, 1966; Gliwicz, 1980; Fons *et al.*, 1997;birds: Crowell & Rothstein, 1981; Blondel, 1985; Wiggins *et al.*, 1998; reptiles: Wharton, 1966; Andrews, 1979; Andren & Nilson, 1983). Our results contribute to a broad, repeatable ecological generality for
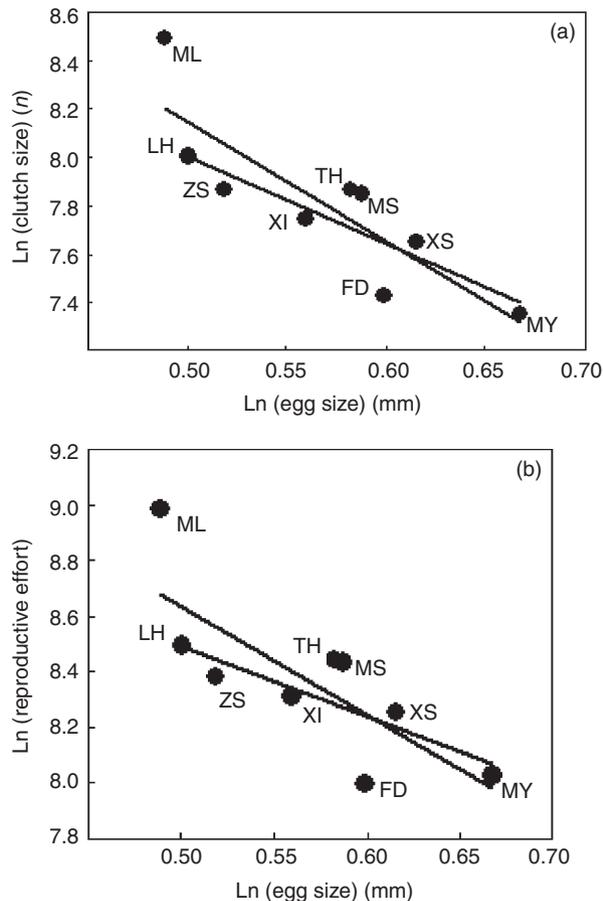
Figure 3 Relationships between (a) egg size and clutch size for islands alone ($r=-0.832$, $n=8$, $P=0.010$) and for islands and mainland combined ($r=-0.854$, $n=9$, $P=0.003$); (b) egg size and reproductive effort for islands alone ($r=-0.719$, $n=8$, $P=0.044$) and for islands and mainland combined ($r=-0.785$, $n=9$, $P=0.012$). ML, Mainland; ZS, Zhoushan; LH, Liuheng; TH, Taohua; XS, Xiushan; MS, Meishan; XI, Xiashi; FD, Fodu; MY, Mayi.

Table 3 Multiple regression analysis, ln (clutch size) $=a+b1$ (ln egg size) $+b2$ (ln body size), to examine the relationship between clutch size and egg size, controlled for size for (a) islands alone and (b) islands and mainland combined

Variable	Coefficient	t-ratio	P
(a) Islands alone			
Constant	5.229	1.438	0.210
ln (Egg size)	−0.815	−3.145	0.026
ln (Body size)	0.494	1.271	0.260
(b) Islands and mainland combined			
Constant	4.726	0.938	0.385
ln (Egg size)	−0.798	−3.241	0.018
ln (Body size)	0.433	1.176	0.284

Table 4 Multiple regression analysis, ln (reproductive effort) $=a+b1$ (ln egg size) $+b2$ (ln body size), to examine the relationship between reproductive effort and egg size, controlled for size for (a) islands alone and (b) islands and mainland combined

Variable	Coefficient	t-ratio	P
(a) Islands alone			
Constant	4.845	1.321	0.244
ln (Egg size)	−0.758	−2.597	0.048
ln (Body size)	0.518	1.356	0.233
(b) Islands and mainland combined			
Constant	4.329	0.843	0.431
ln (Egg size)	−0.752	−2.797	0.031
ln (Body size)	0.449	1.229	0.265

insular shifts in life-history traits across a range of terrestrial vertebrate taxa.

Egg size was traded off against clutch size and was negatively correlated with total reproductive effort for islands alone and for mainland and islands combined, even after controlling for the effects of body size. Several studies have reported negative correlations between egg size and number (Fox, 1997; Fischer *et al.*, 2006; Warne & Charnov, 2008). Our study provides the first evidence for the trade-off between egg size and clutch size among frog populations inhabiting a large number of islands. The negative relationship between total reproductive effort and egg size supports the model of Winkler & Wallin (1987) and is in accordance with earlier artificial selection experiments and comparative analyses (Schwarzkopf *et al.*, 1999; Caley *et al.*, 2001; Czesak & Fox, 2003; but see Fischer *et al.*, 2006; Karl *et al.*, 2007).

The insular shifts in life-history traits in pond frogs may result from different selection pressures on islands. Compared with mainland populations, pond frogs in the Zhoushan Archipelago are confronted with lower numbers of predator species (Huang, 1990; Zhu, 1990; Zhuge, 1990), increased food availability (Wu *et al.*, 2006) and increased population density (Wang, 2006). A decrease in predator pressure often increases the parents' probability of survival, which leads to high population densities. Models predict this would lead to a decrease in the reproductive effort of the parents (Michod, 1979; Shine & Schwarzkopf, 1992). Increased food availability on islands may also lead to increased frog densities (Wu *et al.*, 2006), which in turn selects for larger body size, larger egg size and reduced total reproductive effort (Stamps & Buechner, 1985; Adler & Levins, 1994).

Higher population densities on islands lead to a more intense intra-specific competition among individuals and large-sized individuals are competitively superior under such conditions (Angerbjörn, 1986; Adler & Levins, 1994; Robinson-Wolrath & Owens, 2003). Higher densities of pond frogs also favor the evolution of a larger egg size because offspring from larger eggs reach metamorphosis faster (and at a comparatively larger body size) and have higher fitness compared with offspring from smaller eggs (Berven & Chadra, 1988; Kaplan, 1992; Dziminski & Roberts, 2006). Life-history theories suggest that there is a trade-off between reproduction and growth or maintenance (Roff, 1992; Stearns, 1992). A reduction in reproductive effort will favor growth or maintenance. On the islands,

pond frogs allocating fewer resources to reproduction may assign more resources to growth or maintenance, which results in a larger body size on the islands. Such negative relationships between reproductive effort and body size are also found in mammals and reptiles (Brown & Sibly, 2006; Warne & Charnov, 2008).

Our study suggests that reproductive effort and egg size, rather than reproductive effort and clutch size, are more likely selected mainly for two reasons. First, reproductive effort and egg size are tightly linked in the model of Winkler & Wallin (1987), and offspring from larger eggs reach metamorphosis faster and have higher fitness compared with offspring from smaller eggs (Kaplan, 1992; Dziminski & Roberts, 2006). Second, under high density conditions on islands, the trade-off between number and size of offspring will shift to larger offspring with improved survival because life histories become increasingly sensitive to changes in the juvenile mortality rate (Stearns, 1992), and accordingly clutch size is under indirect selection. Therefore, reproductive effort and egg size related more directly to fitness than reproductive effort and clutch size.

Our results suggest that island area has significant effects on life-history traits in pond frogs. We found that clutch size and reproductive effort of pond frogs increased with island area, while egg size and body size decreased with island area. Such shifts in life-history traits could possibly be attributed to the predicted changes in resource availability, predation and population density with island area (Adler & Levins, 1994). No association between isolation distance and life-history traits may be due to the fact that pond frogs cannot disperse among the islands and mainland (Yiming *et al.*, 1998).

Phenotypic changes in the total reproductive effort, egg size, clutch size and body size observed among pond frogs on islands may partly reflect genetic changes as well as simple phenotypic plasticity in these traits. This can be seen from shifts in clutch size between the islands and the mainland. Larger females in a population usually produce larger clutches than smaller ones because larger females can afford to allocate more resources to reproduction than smaller females (Cummins, 1986; Andersson, 1994). If shifts in clutch size for pond frogs on the islands result solely from adaptive phenotypic plasticity, it would be expected that clutch size would correlate positively with female body size on the islands, or that clutch size on some islands where females are bigger should be larger than the mainland. Contrary to these expectations, clutch size was not associated with body size on islands (Fig. 4) and clutch size on all islands sampled was smaller than on the mainland (Table 2). Therefore, the usual relationship between clutch size and body size on the mainland has been broken (Fig. 4), which suggests that pond frogs on islands probably have evolved genetic mechanisms for directly controlling changes in clutch size. Owing to the long time of isolation of the Zhoushan Archipelago for about 7000–9000 years (Zhou, 1987), sufficient genetic variation may be available in pond frog populations on the islands for directional selection to proceed. This directional selection is
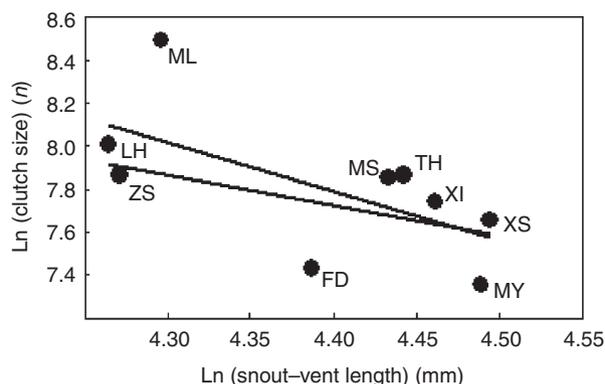
Figure 4 Relationships between clutch size and female snout–vent length of pond frogs *Rana nigromaculata* for islands alone ($r = -0.596$, $n = 8$, $P = 0.119$) and for islands and mainland combined ($r = -0.627$, $n = 9$, $P = 0.070$). ML, Mainland; ZS, Zhoushan; LH, Liuheng; TH, Taohua; XS, Xiushan; MS, Meishan; XI, Xiashi; FD, Fodu; MY, Mayi.

the difference in density among islands and the mainland (Wu *et al.*, 2006).

Acknowledgments

References

Adler, G.H. & Levins, R. (1994). The island syndrome in rodent populations. *Q. Rev. Biol.* **69**, 473–490.

Andersson, M. (1994). *Sexual selection*. New Jersey: Princeton University Press.

Andren, C. & Nilson, G. (1983). Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. *Amphibia-Reptilia* **4**, 63–79.

Andrews, R.M. (1979). Evolution of life histories: a comparison of *Anolis* lizards from matched island and mainland habitats. *Brevoria* **454**, 1–51.

Angerbjörn, A. (1986). Gigantism in island populations of wood mice (*Apodemus*) in Europe. *Oikos* **47**, 47–56.

Berven, K.A. & Chadra, B.G. (1988). The relationship among egg size, density and food level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* **75**, 67–72.

Blondel, J. (1985). Breeding strategies of the blue tit and coal tit (*Parus*) in mainland and island Mediterranean habitats: a comparison. *J. Anim. Ecol.* **54**, 531–556.

Brown, J.H. & Sibly, R.M. (2006). Life-history evolution under a production constraint. *Proc. Natl. Acad. Sci. USA* **103**, 17595–17599.

Caley, M.J., Schwarzkopf, L. & Shine, R. (2001). Does total reproductive effort evolve independently of offspring size? *Evolution* **55**, 1245–1248.

Chen, Q.Y. (1989). *The annals of current and historical place names in Zhejiang Province*. Hangzhou: Zhejiang Education Press (in Chinese).

Crowell, K.L. & Rothstein, S. (1981). Clutch sizes and breeding strategies among Bermudan and North American passerines. *Ibis* **123**, 42–50.

Cummins, C.P. (1986). Temporal and spatial variation in egg size and fecundity in *Rana temporaria*. *J. Anim. Ecol.* **55**, 303–316.

Czesak, M.E. & Fox, C.W. (2003). Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution* **57**, 1121–1132.

Darlington, R.B. & Smulders, T.V. (2001). Problems with residual analysis. *Anim. Behav.* **62**, 599–602.

Dziminski, M.A. & Roberts, J.D. (2006). Fitness consequences of variable maternal provisioning in quacking frogs (*Crinia georgiana*). *J. Evol. Biol.* **19**, 144–155.

Fei, L. (1999). *Atlas of amphibians of China*. Zhengzhou: Henan Science and Technology Publishing House (in Chinese).

Fischer, K., Bot, A.N.M., Brakefield, P.M. & Zwaan, B.J. (2006). Do mothers producing large offspring have to sacrifice fecundity? *J. Evol. Biol.* **19**, 380–391.

Fitch, H.S. (1985). Variation in clutch and litter size in New World reptiles. *Univ. Kans. Mus. Nat. Hist. Misc. Publ.* **76**, 1–76.

Fons, R., Francoise, P., Josette, C. & Henri, C. (1997). Decrease in litter size in the shrew *Crocidura suaveolens* (Mammalia, Insectivora) from Corsica (France): evolutionary response to insularity? *Can. J. Zool.* **75**, 954–958.

Fox, C.W. (1997). Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. *Can. J. Zool.* **75**, 1465–1473.

Freckleton, R.P. (2002). On the misuse of residuals in ecology: regression of residuals vs. multiple regression. *J. Anim. Ecol.* **71**, 542–545.

García-Berthou, E. (2001). On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J. Anim. Ecol.* **70**, 708–711.

Gliwicz, J. (1980). Island populations of rodents: their organization and functioning. *Biol. Rev.* **55**, 109–138.

Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.

Huang, M.H. (1990). *Fauna of Zhejiang: Amphibia and Reptilia*. Hangzhou: Zhejiang Science and Technology Publishing House (in Chinese).

Jewell, P.A. (1966). Breeding season and recruitment in some British mammals confined on small islands. *Symp. Zool. Soc. Lond.* **15**, 89–116.

Kaplan, R.H. (1992). Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* **73**, 280–288.

Karl, I., Lorenz, M.W. & Fischer, K. (2007). Energetics of reproduction: consequences of divergent selection on egg size, food limitation, and female age for egg composition and reproductive effort in a butterfly. *Biol. J. Linn. Soc.* **91**, 403–418.

Lips, K.R. (2001). Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a neotropical treefrog. *Oecologia* **128**, 509–518.

Michod, R.E. (1979). Evolution of life histories in response to age-specific mortality factors. *Am. Nat.* **113**, 531–550.

Nussbaum, R.A. (1984). Amphibians of Seychelles. In *Biogeography and ecology of the Seychelles Islands*: 379–416. Stoddart, D.R. (Ed.). Boston: Dr W Junk Publishers.

Pough, F.H., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitzky, A.H. & Wells, K.D. (1998). *Herpetology*. New Jersey: Prentice-Hall.

Ricklefs, R.E. (1980). Geographical variation in clutch-size among passerine birds: Ashmole's hypothesis. *Auk* **97**, 38–49.

Robinson-Wolrath, S.I. & Owens, I.P.F. (2003). Large size in an island-dwelling bird: intraspecific competition and the dominance hypothesis. *J. Evol. Biol.* **16**, 1106–1114.

Roff, D.A. (1992). *The evolution of life histories: theory and analysis*. New York: Chapman & Hall.

Ryser, J. (1989). Weight loss, reproductive output, and the cost of reproduction in the common frog, *Rana temporaria*. *Oecologia* **74**, 264–268.

Schwarzkopf, L., Blows, M.W. & Caley, M.J. (1999). Life-history consequences of divergent selection on egg size in *Drosophila melanogaster*. *Am. Nat.* **154**, 333–340.

Shine, R. & Schwarzkopf, L. (1992). The evolution of reproductive effort in lizards and snakes. *Evolution* **46**, 62–75.

Smith, C.C. & Fretwell, S.D. (1974). The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499–506.

Smith, R.J. (1999). Statistics of sexual size dimorphism. *J. Hum. Evol.* **36**, 423–459.

SPSS Inc. (1998). *SPSS/PC + 8.0*. Chicago: SPSS Inc.

Stamps, J.A. & Buechner, M. (1985). The territorial defense hypothesis and the ecology of insular vertebrates. *Q. Rev. Biol.* **60**, 155–182.

Stearns, S.C. (1992). *The evolution of life histories*. Oxford: Oxford University Press.

Wang, J.T. & Wang, P.X. (1980). The relationship between sea level rising and climate change on east of China. *Chin. J. Geog.* **16**, 299–313 (in Chinese with English abstract).

Wang, Y.P. (2006). *Study on life history shifts in pond frogs (Rana nigromaculata) and the predatory threats of bullfrogs (Rana catesbeiana) in the Zhoushan Archipelago*. PhD thesis, the Chinese Academy of Sciences.

Warne, R.W. & Charnov, E.L. (2008). Reproductive allometry and the size-number trade-off for lizards. *Am. Nat.* **172**, E80–E98.

Wharton, C.H. (1966). Reproduction and growth in the cottonmouths, *Agkistrodon piscivorus* Lacepede, of Cedar Keys, Florida. *Copeia* **1966**, 149–161.

Wiggins, D.A., Møller, A.P., Sørensen, M.F.L. & Brand, L.A. (1998). Island biogeography and the reproductive ecology of great tits *Parus major*. *Oecologia* **115**, 478–482.

Winkler, D.W. & Wallin, K. (1987). Offspring size and number: a life history model linking effort per offspring and total effort. *Am. Nat*. **129**, 708–720.

Wu, Z.J., Li, Y. & Murray, B.R. (2006). Insular shifts in body size of rice frogs in the Zhoushan Archipelago, China. *J. Anim. Ecol*. **75**, 1071–1080.

Yiming, L., Niemelä, J. & Dianmo, L. (1998). Nested distribution of amphibians in the Zhoushan Archipelago, China: can selective extinction cause nested subsets of species? *Oecologia* **113**, 557–564.

Yiming, L., Zhengjun, W. & Duncan, R.P. (2006). Why islands are easier to invade: human influences on bullfrog invasion in the Zhoushan Archipelago and neighboring mainland, China. *Oecologia* **148**, 129–136.

Yuan, C.M. (1965). The preliminary investigation of amphibians on Zhoushan, Putuo and Taohua. *Chin. J. Zool*. **7**, 114–116 (in Chinese).

Zhou, J.L. (1987). *The comprehensive agriculture programme of Zhoushan City*. Hangzhou: Zhejiang People's Press (in Chinese).

Zhu, X. (1990). Ecological birds geography of Zhoushan Archipelago. *J. Zhejiang Forestry Coll*. **7**, 153–160 (in Chinese with English abstract).

Zhuge, Y. (1990). *Fauna of Zhejiang: Mammalia*. Hangzhou: Zhejiang Science and Technology Publishing House (in Chinese).