Personality of a clonal gecko *Lepidodactylus lugubris*: developmental mechanism and relation to microhabitat use

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クローン種ヤモリにおける個性の発達形成および微環境利用との関連

酒井理

多くの動物種では探索行動や捕食者回避行動に個体差があり、各個体の傾向は時間や状況を越えて一貫する。このような現象は個性として捉えられ、その形成過程や適応的意義を理解する試みが近年の行動学において盛んにおこなわれてきた。個性の形成において遺伝的要因が寄与する割合は半分程度と考えられており、後天的要因が与える影響の理解が求められている。また、行動傾向の異なる個体の自然下での振る舞いを明らかにすることは、個性の適応的意義の理解に繋がる。オガサワラヤモリは２倍体や３倍体の多様なクローンの集団が自然下に生息するという点で脊椎動物において稀有な例であり、個々のクローン集団は後天的要因による個性の形成や適応的意義を探求するのに適した対象である。本研究では本種の単一クローンから成る野外集団と、同じく単一クローンに属する飼育個体を用いて、個性の発達形成のメカニズムを解明し、また、個性と生息微環境との関連性を明らかにすることを目指した。

クローン動物では先天的な個体差が僅かで、各個体が異なる経験を重ねることで多様性が生じていくと予想される。主論文１では発達段階を考慮して野外集団の個性の有無と多様性を調べた。幼体と成体ともにリスク回避傾向と探索傾向に一貫した個体差がみられ、自然下では発達にともない個性のバリエーションが増加する傾向があることが明らかとなった。

行動傾向は自然下での空間利用パターンに影響することが知られており、クローン動物においても個性と生息環境が関連することが予想される。主論文２では海岸植生の低木林に生息する集団を対象とし、標識再捕獲法を用いて本種の空間利用パターンを調べ、各個体の行動傾向と生息微環境との関連性を調べた。その結果、本種は林内でも特定の樹種に局在し定住性が高いこと、また、大胆な個体は林内の全域に生息するが臆病な個体は道路側に面した低い枝にのみ生息していることが判明した。

栄養状態の差が行動傾向の差を生じさせ、発達を通して徐々に個性の多様化を促進するという仮説が提唱されている。主論文３ではこの仮説の検証を目的とし、孵化から性成熟までの 15 カ月間にわたって給餌量を制御してヤモリを飼育し行動形質の変化を観察した。給餌量の差は成長速度と繁殖開始齢に顕著な影響を与えたが、活動性や採餌の積極性には影響しなかった。リスク回避傾向にのみ給餌処理の影響がみられたが、幼体時に急激に生じた多様化は成長に伴い消失していった。したがって本種では、生活史形質やエネルギー貯蔵量が行動形質とは相関せず、発達を通した個性の多様化を予測する既存の仮説は支持されなかった。また、リスク回避傾向の一時的な多様化とその後の収束がみられたことから、発達に伴う捕食リスクや採餌能力の変化によって、栄養状態が行動形質に与える影響の仕方も変化する可能性が示唆された。

本研究は、自然下のクローン動物において後天的に形成される個性が発達にともない多様化すること、また、行動傾向と生息微環境が関連することを初めて示した例となる。しかし、個性の形成メカニズムは既存の仮説よりも複雑であり、後天的要因によって行動形質の多様化が生じる過程を理解するには、発達段階によって優勢となるメカニズムが異なることを考慮した新たな概念が重要であると提起された。

Personality of a clonal gecko *Lepidodactylus lugubris*: developmental mechanism and relation to microhabitat use

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INTRODUCTION

Over the past few decades, there have been a growing appreciation for the importance of behavioural variation within a species and behavioural consistency within individuals through time and across situations. These consistent behavioural differences among individuals are described as personality and have been reported from a wide range of taxonomic groups. With accumulating evidence of animal personality, researchers have attempted to understand how and why behavioural variation is generated and maintained.

From a proximate perspective, personality is formed by interactions between genetic and environmental factors. Environmental factors seem to be very important for contribute to a personality formation, but few empirical studies have investigated its effects because of the difficulty in separating from genetic factors. From an ecological perspective, behavioural tendencies are seems to be associated with individual differences in habitat use and dispersal patterns. Ecological consequences of personality are therefore implied from information of how individuals with different characteristics live in natural environments. However, in order to understand the general principle, further case studies of free-living animal species are necessary.

Using clonal animals is a powerful approach to reveal personality formation because we can extract the effects of non-genetic factors on phenotypic variation. *Lepidodactylus lugubris* is an obligate parthenogenetic gecko, which reproduces genetically uniform individuals without mating. The unique point of *L. lugubris* is its existence of wild-living clonal populations. Thus, it is a suitable subject for investigating personality formation and its ecological relationship. In the present study, using wild and captive *L. lugubris*, I aimed to reveal the developmental mechanisms of personality formation and relation between spatial ecological features and behavioural tendencies.

MATERIALS AND METHODS

First, assuming that innate individual differences among clones are minimal, I expected that accumulating experiences of each individual would generate variation throughout the ontogeny. Thus, I investigated the presence of personality in free-living clonal geckos at different developmental stages. I repeatedly measured exploration and risk-taking tendencies to assess behavioural consistency. In addition, I compared variation of these behavioural traits between juveniles and adults.

Second, if clonal geckos have personality, their behavioural tendencies might be associated with spatial ecological features. In order to investigate their spacing and movement patterns, I conducted a mark-recapture survey for a population in a small beachfront forest facing two contrasting environments with respect to

human disturbance. Based on the field survey and behavioural tests, I examined possible association between microhabitat use and behavioural tendency.

Third, several hypotheses predict that mutual interactions between intrinsic state and behaviour gradually generates, or sometimes diminishes, behavioural variation with ontogeny. However, these models should be empirically tested with a simple study system. Here, using laboratory hatched and raised clonal geckos, I investigated effects of energy intake on behavioural traits and challenged to reveal a relative applicability of two previously proposed models: pace-of-life syndrome (POLS) and asset protection. I controlled food supply of two groups (high or low energy intake) and evaluated some of their life-history traits and behavioural traits from hatching to sexual maturation.

RESULTS AND DISCUSSION

In a wild population, each individual exhibited different exploration and boldness with significant repeatability in three consecutive trials, indicating the presence of personality in clonal geckos. In addition, adults showed a larger behavioural variation than juveniles, suggesting that appearance of different behavioural types during their ontogeny increased the personality variation within a clonal population.

With regards to spacing patterns, geckos exhibited a highly site fidelity, small range of movement, and consistency of perch height at an individual level. Relatively shy geckos were captured from only low branches on road side trees, whereas relatively bold geckos were captured from any microhabitats within the forest. Given that the field site faces two different environments, the presence/absence of artificial disturbance may influence boldness of resident geckos via personality formation or microhabitat selection.

In the laboratory experiment, the different amounts of energy intake induced differences in growth rate and reproduction but not in foraging-boldness and activity. The exception was risk-boldness; the high energy group maintained to be bold throughout the development, whereas the low energy group changed to be shy at early stages and returned to be bold at later stages. These results did not support the predictions proposed by the POLS and asset protection models. Considering that predation risk and food availability change with the development of geckos, the state-dependent safety model could explain divergence and convergence of risk-taking propensity at their different life stages.

CONCLUSION

The present study demonstrates the significance of non-genetic factors in personality formation and association between microhabitat use and behavioural tendency in free-living clonal animals. However, developmental mechanisms of personality formation seem to be more complicated than the predictions made by the simple current models. This study also highlights the importance of “life stage specific” effect of state on behaviour for further understanding of personality formation.

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ARTICLE

Comparison of personality between juveniles and adults in clonal gecko species

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Abstract
The developmental perspectives of animal personality enhance our understanding of how personality structure changes in relation to life stage. Clonal animals are ideal models for developmental studies because personality differences can be solely attributed to environmental factors. Here, I investigated the presence of personality within a species of clonal gecko, *Lepidodactylus lugubris*, at different developmental stages. For juveniles and adult geckos, I measured exploration (reaction to a novel situation) and boldness (risk-prone tendency) and evaluated repeatability and correlation of these behavioural traits. Each gecko exhibited different exploration and boldness with significant repeatability through time but no correlation between these behavioural traits. Small juveniles were composed of only bold and low explorative individuals but large juveniles and adults were composed of various personality type individuals. These results demonstrate that subject geckos have a similar personality structure across life stages and that exploration and boldness are independent personality without forming behavioural syndrome structure. Biased composition of personality type between life stages suggests that appearance of different personality type individuals during an early ontogenetic stage generates personality variation within the clonal population. This study provides developmental insight about personality structure and its composition in clonal animals living in the wild.

Keywords Animal personality · Developmental stage · Clonal animal · Parthenogenetic gecko · *Lepidodactylus lugubris*

Introduction

Consistent behavioural differences between individuals are described as animal personality, and this topic is a burgeoning field of study in behavioural ecology and ethology (Gosling 2001; Réale et al. 2007; Carere and Maestripieri 2013). Animal personality is reflected by consistency of a single behavioural trait through time (repeatability) and across situations, and by correlations between multiple behavioural traits in different contexts (behavioural syndromes) (Sih et al. 2004; Bell et al. 2009). Several studies have reported that animals in a wide range of taxa have personality, demonstrating its ubiquitous nature in animals (Smith and Blumstein 2008; Bell et al. 2009; Garamszegi et al. 2012).

The ontogeny of animal personality may enhance our understanding of its function and evolution because adaptive behavioural responses might change with different demands and selective pressures at different life stages (Stamps and Groothuis 2010; Groothuis and Trillmich 2011). In recent years, researchers have demonstrated that several animal species have personality structure that may change across life stages (Petelle et al. 2013; Guenther et al. 2014; Class and Brommer 2015; Wuerz and Krüger 2015) and that personality type is not always stable throughout an individual's life span (Müller and Müller 2015; Favati et al. 2016; Wexler et al. 2016). These findings suggest that animal personality changes across life stages that are associated with life-history dependent behavioural strategies.

An ideal approach to clarify the developmental mechanisms of personality is to use "replicate individuals" which are genetically identical to one another (Stamps and Groothuis 2010). In practice, clonal animals are suitable subjects as the replicate individuals because of their genetic uniformity (Vrijenhoek 1994; Schuett et al. 2011). If individuals with the same genotypes are used for developmental

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studies, we no longer have to consider the differences of genetic factors among individuals and so can attribute differences among individuals solely to environmental factors (Lewejohann et al. 2011; Freund et al. 2013). Therefore, using clonal animals is a simple and powerful approach to investigate the ontogeny of personality, but there are few studies that have dealt with the development of personality in clonal animals.

In reptiles, approximately 40 species exhibit parthenogenesis (Kearney et al. 2009). They reproduce full clonal individuals without copulation and fertilization, and exclusively female populations exist in the wild. The mourning gecko, *Lepidodactylus lugubris*, is an obligate parthenogenetic reptile species that shows a wide distributional range throughout tropical and subtropical areas in the Pacific, Asia, and South America (Moritz et al. 1993; Ineich 1999). Although this species includes various diploid and triploid clonal lineages with different genetic components (Ineich 1988; Ineich and Ota 1992; Radtkey et al. 1995), genetic uniformity is evident within a single clonal lineage (Yamashiro et al. 2000; Wilmhoff et al. 2003). Mourning geckos are solitary throughout their lifetime, and mothers display no parental care, which minimizes the influence of maternal effects through the interaction between mothers and juveniles. Thus, given its genetic uniformity and solitary life history, *L. lugubris* is an ideal subject for personality studies from a developmental perspective.

Here, I investigated the presence of personality in clonal mourning geckos, *L. lugubris*, at different developmental stages. Using juvenile and adult wild-caught geckos, I conducted behavioural tests repeatedly and measured exploration and boldness to assess behavioural repeatability (consistent behavioural tendencies through time) and behavioural syndrome structure (correlation between exploration and boldness). I then compared absolute values of exploration and boldness between juveniles and adults by considering several morphological and body condition factors. This allowed me to assess the effect of developmental stage and individual body condition on their behavioural trait.

Materials and methods

Subjects

The mourning geckos were collected from a small beachfront forest located in Okinawa-jima Island, Japan (26°44′53.6″N, 128°10′46.7″E). Populations in this area were introduced in the latter half of the twentieth century and so are assumed to be recently founded populations (Ota et al. 2004). Although this species includes several clonal lineages, only a single clonal lineage (C-type clone) is distributed in the study area (Yamashiro et al. 2000). Each clonal lineage of the species can be distinguished by their dorsal dot patterns (Ineich 1988; Ineich and Ota 1992). In addition, molecular analyses supported genetic uniformity within C-type clones and validity of classification based on the dorsal dot pattern (Yamashiro et al. 2000; Wilmhoff et al. 2003; Murakami et al. 2015). Only individuals with the dorsal dot pattern unique to C-type clones were used in this experiment.

A total of four capture surveys were carried out (September 2013, June 2014, September 2014, and September 2015). Each capture period lasted for approximately 20 days. Mourning geckos are nocturnal and so on each day, I started to capture geckos at sunset and continued until midnight. Geckos were captured by hand and placed in small Ziploc bags (10 × 7 cm) which had been punctured with several small holes to allow air exchange. Captured geckos were immediately transported to an indoor field-laboratory close (< 1 km) to the beachfront forest from where they were taken. The day following capture, I measured snout to vent length (SVL), body mass, tail condition (whether tail was original or regenerated), and gravid condition (the presence of visible eggs observed through abdomen) of geckos. Except during the measurement process and behavioural tests, geckos were kept in the Ziploc bags and placed in the room of the laboratory with natural light. I did not provide food or water for geckos during the 4 days they were captive. After the experiment, geckos were marked by toe-clipping to identify individuals, and released immediately in the vicinity of where they were found. This individual marking was aimed not only at the present study but was also for other studies investigating the population ecology of the focal geckos.

Determination of developmental stage

Maturation size of the mourning geckos was defined based on SVL of gravid females captured from the focal population. Out of 301 individuals collected from this field site over 3 years, the minimum SVL of a gravid female was 36.8 mm (Sakai 2016). Therefore, an SVL of 36.8 mm was set as a borderline between juveniles and sexually mature adults, which is close to values reported in other populations of this species (35.0 mm; Ota 1994). I collected and tested juveniles only in the autumn, because juvenile sized geckos were absent in the focal population in early summer (Sakai 2016).

It should be noted that several age class individuals were included in each developmental stage of the present study. Hatchlings of the C-type clones of mourning geckos are 18.0 ± 1.1 mm in SVL (mean ± SD, $N = 97$) and it takes approximately 10 months for individuals to reach sexual maturity in the wild and in laboratory populations (Sakai, unpublished data). In addition, the life span of mourning geckos is at least 3 years and sometimes over 5 years in

captivity (Sakai, unpublished data). Thus, in the present study, the juvenile stage has age range of several months and the adult stage has an age range of several years.

Behavioural tests

First, I aimed to examine a key feature of personality (behavioural repeatability) (Réale et al. 2007; Carere and Maestripieri 2013). Second, I was also interested in whether there was a correlation between different kinds of behavioural trait (behavioural syndrome) (Sih et al. 2004). To achieve the above aims, the experiment was designed to conduct the same behavioural tests 3 times, each including two separate assays for exploration and boldness. I conducted one behavioural test on three consecutive nights, with the first test starting the night following capture. Over the four survey periods, I sometimes recaptured individuals that had been previously used for the behavioural tests. In these cases, I took these individuals back to the laboratory and repeated the same three tests as I conducted when they were originally captured. I refer to these as retests, and this allowed me to evaluate test–retest repeatability of the behavioural traits.

All experiments were conducted in the laboratory at night in darkness because the mourning gecko is nocturnal. Air temperature in the room was controlled at 27.9 ± 1.2 °C (mean ± SD, $N = 909$ recordings) for all tests. I recorded the behaviour of the geckos using a Sony digital video camera (HDR-XR550V) set to night vision mode. The experimental arena was a plastic container (12 × 20 × 12 cm), in which grid lines (4 × 4 cm) had been drawn on the walls and floor. A refuge made of cardboard (5 × 5 × 1.5 cm) was set at the centre of the arena's floor. I cleaned the inside of each container with wet tissue paper after I used it for each trial. After each trial had ended, the subject was placed back into the Ziploc bag where they were being kept.

Novel environment assay for exploration

Subjects were acclimated in the Ziploc bags in the dark experimental room for at least 30 min before tests. After acclimation, I turned on the light and gently introduced a gecko from the bag into the experimental arena without touching it with my hands (in order to minimize a threat stimulus assumed to be a predatory attack). I immediately turned the light off again and left the room and I recorded the gecko's behaviour for the next 40 min with the video camera.

Exploration is defined as the reaction to a novel environment or situation (Réale et al. 2007; Carter et al. 2013). The experimental arena was a potentially novel environment for wild geckos; therefore, in this assay the number of blocks that the gecko moved into was counted as the indicator. Because geckos sometimes hid within a refuge or walked on the lid of the container which had no grid lines drawn on it, a relative exploration score was calculated by the following two steps. First, I calculated the total exploration duration when geckos were out of the refuge and the lid. Second, I divided the number of blocks in the arena that the gecko had moved by the total exploration duration (min). According to this calculation, the relative exploration tendency per minute of each subject with a different exploring duration could be evaluated, and a higher score was associated with being more explorative.

Threat stimulus assay for boldness

Immediately after the novel environment assay, I entered the experimental room and turned the light on. I proceeded to chase the gecko around the arena by hand to induce it hide into the refuge. Although chase time depended on how quickly the subject entered into the refuge (e.g., it took at least 10 s and at most 60 s), I standardized the situation that geckos voluntarily run away into the refuge. I immediately turned the light off and left the room and I recorded the gecko's behaviour for a further 20 min.

Boldness is defined as an individual's risk avoidance tendency (Wilson et al. 1994; Réale et al. 2007). The chase stimulus was a threat experience which the gecko would perceive as being similar to a predatory attack. Therefore, in this assay, hiding duration within the refuge was used as the indicator. The less time that has elapsed since being attacked would be associated with a higher probability that the predator is still present near the refuge when the subject re-emerges. Therefore, individuals that hid for shorter durations before re-emerging from the refuge were considered to be bolder than those which took longer to re-emerge.

Data analysis

A total of 44 juvenile (SVL; mean ± SD = 24.1 ± 3.4 mm) and 41 adult (43.1 ± 2.7 mm) geckos were used for the behavioural tests. First, I investigated the effect of developmental stage on behavioural traits, so I analysed boldness and exploration for all individuals with univariate linear mixed-effect models (LMMs) (Dingemanse and Dochtermann 2013). Developmental stage (juvenile or adult) was set as a fixed factor, and individual identity and year (2013, 2014, and 2015) were fitted as random factors. Second, I examined the effect of individual body condition on behavioural traits for each developmental stage separately. In LMMs, the night of test (1st, 2nd, and 3rd night), body length (SVL mm), and tail condition (original or regenerated tail) were set as fixed factors. For the adult stage, I also added gravid condition (egg presence or absence) as a fixed factor. Individual identity and year (2013, 2014, and 2015) were fitted as random factors for all models. In addition for the adult stage, considering that behavioural tests

were conducted at two seasons, I also added month (June or September) as a random factor. The R package "*lmerTest*" was used for these analyses (Kuznetsova et al. 2015), and a restricted most likelihood *t*-test using Satterthwaite approximations for degrees of freedom was used to determine the significance of fixed factors (see details in Kuznetsova et al. 2015). Behavioural repeatability through three tests was calculated by the ratio of the variance associated with the individual identity to the total phenotypic variance (i.e. sum of individual, yearly and residual variances) based on the above LMMs for juvenile and adult stages separately (Dingemanse and Dochtermann 2013). This repeatability score ranges from 0 to 1, and a higher score indicates higher consistency within individuals compared to total phenotypic variation. A log-likelihood ratio test was used to determine the significance of random factors between models with and without a given random effect.

Test–retest repeatability was evaluated by the correlation of mean behavioural value of the three tests between the original test and the retest using Spearman's rank correlation coefficient. Time intervals between the original and the retest of the same individual should be taken into consideration for repeatability: a relatively long time scale in the context of the subject's life span potentially lowers the consistency of behavioural tendency (Bell et al. 2009). In the present study, the time intervals between two tests varied from short to long periods (mean ± SD = 103.7 ± 154.4 days, range 4–476 days, $N = 27$), so only 16 retests (12 adults and 4 juveniles) with short intervals (mean ± SD = 9.1 ± 3.2 days, range 4–17 days) were used for the analysis. This time scale is short relative to the life history of mourning geckos so we can exclude consideration of their personality change being associated with ontogeny.

I evaluated whether there was a behavioural syndrome between exploration and boldness. The mean value of the three tests was calculated in these behavioural traits, and Spearman's rank correlation coefficient was used for the analysis. All statistical calculations were conducted using the software package R ver. 3.0.2 (R Core Team 2013).

Ethical note

No death, shed tails, or sickness were recorded as a result of the experimental manipulations. Toe clipping is a method that is often used to mark individuals in herpetology research to allow for individual identification. It has been shown to be less stressful than alternative marking methods and it is considered to be the most adequate and ethically sound method for durable marking of small lizards (Perry et al. 2011). I complied with reptile care guidelines and cut one toe per limb, which minimizes the effect on survival or locomotor performance for geckos (Paulissen and Meyer 2000; Hoehn et al. 2015). To prevent infection, I used a pair of sharp surgical scissors that were disinfected with ethanol and flame before and after cutting each toe. Many geckos marked with this technique were also recaptured throughout later field surveys. In all cases, the clipped areas had healed cleanly with no visible sign of infection or adverse effects on body condition.

Results

Effects of developmental stage and individual body condition

The developmental stage significantly affected the absolute value of exploration and boldness scores. Juveniles exhibited a significantly lower exploration score (the number of blocks per minute: median ± SD = 2.90 ± 0.89 times) than adults (4.30 ± 1.06 times) (LMM; $t = -7.01$, $p < 0.001$). In boldness, juveniles were significantly bolder (hiding duration: median ± SD = 2.65 ± 4.40 min) than adults (5.30 ± 4.37 min) ($t = -2.27$, $p = 0.026$).

Individual body condition was related to exploration score, but there were significant differences between juveniles and adults (Table 1). SVL was positively correlated with exploration score in juveniles, but not in adults (Fig. 1). Adults with regenerated tails had higher exploration scores than those with original tails, but this tendency was not detected in juveniles. For boldness, no significant effect of individual body condition was detected in either developmental stage (Table 1). However, breaking the juveniles into two groups (small SVL < 22 mm; large 22 mm < SVL < 36.8 mm), there was a relationship between SVL and boldness score. The small juvenile group was composed of only bolder individuals which showed short hiding duration (< 5 min), whereas the large juvenile group and adults had more variation in their boldness (Fig. 1).

Behavioural repeatability and syndrome structure

In repeatability through the three consecutive tests, geckos in both developmental stages exhibited significant consistency in the two behavioural traits (Table 2). Exploration scores were repeatable in juveniles (ratio = 0.194) and adults (ratio= 0.277), and boldness scores were repeatable in juveniles (ratio = 0.436) and adults (ratio = 0.319). The random yearly factor significantly related to exploration in the juvenile stage, but not to boldness in either developmental stage.

When I examined test–retest repeatability, the mean boldness score was significantly correlated between the original test and the retest ($r_s = 0.694$, $p = 0.003$, $N = 16$). Mean exploration score of the original test was marginally correlated with the score of the retest ($r_s = 0.481$, $p = 0.059$, $N = 16$). Finally, there was no significant correlation

Table 1 Effect of individual body condition on exploration and boldness scores of mourning geckos

Behavioural trait	Fixed factor	Estimate	SE	*t*	*p*
Juvenile stage					
Exploration	Night of test	0.04	0.08	0.57	0.569
	Snout-vent length	0.16	0.03	6.18	**< 0.001**
	Tail condition (regenerated)	0.06	0.20	0.30	0.766
Boldness	Night of test	− 0.31	0.42	-0.74	0.461
	Snout-vent length	0.29	0.20	1.48	0.147
	Tail condition (regenerated)	0.25	1.52	0.16	0.872
Adult stage					
Exploration	Night of test	− 0.13	0.10	-1.30	0.197
	Snout-vent length	0.00	0.06	0.07	0.944
	Tail condition (regenerated)	0.71	0.29	2.43	**0.020**
	Gravid condition (no eggs)	− 0.12	0.30	− 0.39	0.698
Boldness	Night of test	0.89	0.49	1.81	0.074
	Snout-vent length	− 0.09	0.28	− 0.31	0.758
	Tail condition (regenerated)	− 0.91	1.40	− 0.65	0.520
	Gravid condition (no eggs)	− 2.27	1.45	− 1.56	0.127

All estimates of fixed factors and statistics were derived from univariate linear mixed-effect models for each behavioural trait. Low boldness score indicates increased boldness, whilst high score indicates shyness. "Night of test" represents times that the experimental tests were conducted (1st, 2nd, and 3rd nights). Bold letters represent significant effect ($p < 0.05$)

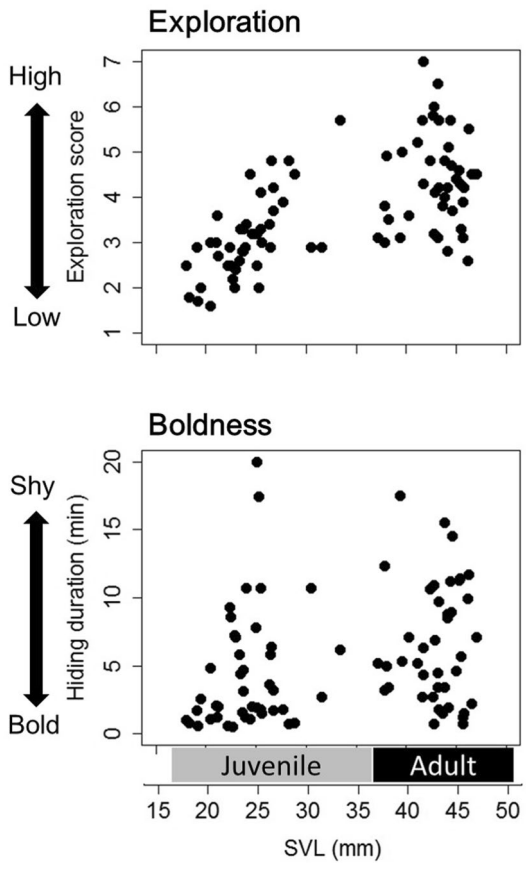

Fig. 1 Exploration and boldness scores of mourning geckos in relation to SVL. Each plot represents mean behavioural value of three tests for each gecko

Table 2 Variance and ratio of random factors on exploration and boldness scores of mourning geckos

Behavioural trait	Random factor	Variance	Ratio	LRT: χ^2	*P*
Juvenile stage					
Exploration	ID	0.161	0.194	6.12	**0.013**
	Year	0.181	0.218	8.43	**0.004**
	Residual	0.488	–	–	–
Boldness	ID	13.315	0.436	22.98	**< 0.001**
	Year	1.777	0.058	0.46	0.499
	Residual	15.450	–	–	–
Adult stage					
Exploration	ID	0.486	0.277	12.65	**< 0.001**
	Year	0.269	0.153	1.49	0.222
	Month	0.174	0.099	0.00	1.000
	Residual	0.827	–	–	–
Boldness	ID	10.832	0.319	11.46	**0.001**
	Year	0.000	0.000	0.00	1.000
	Month	3.175	0.093	0.19	0.663
	Residual	19.998	–	–	–

All estimates of random factors and statistics were derived from univariate linear mixed-effect models for each behavioural trait of each developmental stage. Repeatability is calculated as the ratio by dividing variance associated with ID effect by the total phenotypic variance (sum of variances). LRT represents χ^2 statistics of log-likelihood ratio test, and bold letters represent significant effect ($p < 0.05$)

between exploration and boldness scores in both juveniles ($r_s = -0.023$, $p = 0.88$, $N = 44$) and adults ($r_s = -0.218$, $p = 0.17$, $N = 41$).

Discussion

In exploration and boldness scores, behavioural repeatability was detected in both juvenile and adult stages of mourning geckos, which shows that clonal geckos have personality traits. There was no behavioural correlation between exploration and boldness at either developmental stage. This indicates that juveniles and adults have similar personality structures and that exploration and boldness are independent from one another. This is the first study to report the presence of personality in parthenogenetic reptile species, although it is not the first to examine personality in genetically identical individuals. Previous studies have reported the presence of personality among genetically identical individuals in clonal pea aphids (Schuett et al. 2011, 2014), highly inbred mice (Lewejohann et al. 2011; Freund et al. 2013), and monozygotic human twins (Tellegen et al. 1988).

Most behavioural traits were not explained by individual body condition. The exception was tail condition in adults; geckos with regenerated tails were slightly more explorative than those with original tails (Table 1). Tail autotomy is an anti-predatory behaviour of lizards (Bateman and Fleming 2009). It is considered likely that geckos with regenerated tails have encountered predators in the past, thus a high explorative tendency may be associated with a high predation risk. From another perspective, it should be noted that learning or acclimation to the experiment could change a behavioural tendency because tests were conducted over three consecutive nights. However, the night of the test (1st, 2nd, or 3rd) did not significantly influence boldness or exploration scores in any case (Table 1). This suggests that the repeated measures did not induce a simple trend of learning or acclimation for geckos.

Smaller juveniles were mostly bold with low exploration scores whereas larger juveniles and adults had much more personality variation among individuals (Fig. 1). This biased composition of personality type on small juveniles could have caused the observed differences in boldness and exploration scores between developmental stages. Considering that the body size of juvenile reptiles is associated with age (Andrews 1982), it is likely that large juveniles were several months old and small juveniles were only days or weeks old when I tested them in September. Therefore, the appearance of shy and highly explorative individuals during this early ontogenetic stage may generate the personality variation of clonal geckos. This scenario raises the possibility that different environmental factors acting on each individual may form personalities along different trajectories.

At the group level, the exploration score of juveniles was lower than that of adults, which suggests that their exploration increases with age. The opposite trend has been reported in other vertebrates such as European green lizards, *Lacerta viridis* (Bajer et al. 2015) and red junglefowl, *Gallus gallus* (Favati et al. 2016). These highly explorative juveniles were generally explained by the energy demands needed for growth or migratory demands in order to avoid larger competitors. However, it should be noted that in the present experimental design, size dependent score bias cannot be eliminated because the exploration score was potentially influenced by relative body size to grid size of the arena. A stricter evaluation of exploration score based on relative body size is required for further discussion of exploration specific to developmental stage.

Juvenile geckos also tended to be bolder than adult geckos, suggesting that a risk taking tendency may decline with age. Similar tendencies have been reported in mustard leaf beetles, *Phaedon cochleariae* (Müller and Müller 2015), firebugs, *Pyrrhocoris apterus* (Gyuris et al. 2012), and red junglefowl (Favati et al. 2016). There are a number of explanations for why juveniles might be bolder than adults. First, the size specific predation risk of small juveniles might favour being bolder, which could increase growth. Boldness is positively linked to foraging activity (Mas-Muñoz et al. 2011) and high energy intake accelerates the growth rate of juvenile lizards (Andrews 1982). In small lizard species such as the mourning gecko, small invertebrates (e.g. spiders and centipedes) and other gecko species are potentially risky predators for juveniles (Bauer 1990; Bolger and Case 1992). Therefore, being bold might be a good strategy for juveniles to increase food intake and growth in order to be released from this specific predation pressure. Second, threat experiences may reduce the boldness level in geckos. Encounters with predators may make individuals learn to be shyer (Niemelä et al. 2012; Killen et al. 2013). Longer living individuals potentially have much more experience of encounters with predators than younger individuals, and such accumulated experiences may cause bold geckos to become shyer. Third, selective pressure may eliminate bolder individuals from the focal population. Correlation between survival rate and boldness is reported from various animals (Smith and Blumstein 2008). As a consequence of predation, risk-averse (shy) geckos may survive to an older age than risk-prone (bold) geckos. In order to investigate these possibilities relating to predators, manipulating tests are needed in a controlled environment, with and without predators.

In this study, I demonstrated that juvenile and adult clonal geckos have personality with similar structures. Differences of absolute values of boldness and exploration between developmental stages were detected, which may be due to biased individual composition of small juveniles. These

results show that variation in personality increase during early ontogeny, potentially through the presence of a boldness/growth syndrome or through learning, which may help to understand personality forming throughout ontogeny. From this study, it is unclear whether personality type of individual geckos changes throughout their development, and if it does, what factors influence its change. Therefore, a longitudinal study is needed to clarify the developmental process of personality type within individuals throughout different life stages. It is also unclear whether the observed characteristics (e.g. small juvenile group was composed of only bold individuals) are specific to clonal animals or whether this is also normal in sexually reproducing taxa. I therefore advocate a comparative approach to studying the personality structure, variation, and absolute value of behavioural traits between clonal species and related sexual species in order to reveal the impact of genetic and environmental factors on the formation of personality.

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TITLE PAGE

Behavioural tendency associated with microhabitat use in a clonal gecko species living in the wild

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Abstract

Ecological consequence of individual variation is implied from information how individuals with different characteristics live under nature. Individual variation in behavioural traits is described as animal personality and has been reported even from genetically identical animals. Considering that animal personality influences spacing and movement patterns, it can be expected that behavioural tendencies of clonal individuals are associated with their spatial ecological features in the wild. Here, I investigated relationship between personality of a clonal gecko *Lepidodactylus lugubris* and its space use. The study site is a small beachfront forest facing two contrasting environments with respect to human disturbance. I conducted a mark-recapture survey to assess a gecko's spacing pattern and quantified their boldness and exploration scores. Geckos exhibited a small range of movement, a high level of site fidelity at the same trees, and consistency of perch height; some geckos were repeatedly collected from low branches but others were from high branches. Relatively shy geckos were collected from only low branches on road side trees, whereas relatively bold geckos were collected from a wide range of microhabitats. These findings suggests that some geckos do not habituate but sensitize to human disturbance, and that different microhabitats may shape different personality types throughout the developmental plasticity.

Significance statement

Individuals within conspecifics show different spacing and movement patterns

and such features are partly associated with their behavioural tendencies. Clonal animals have personality despite their genetic uniformity, and if they are exposed to a spatially diverse environment, different personality types may exhibit different space use. Here, I examined possible association of behavioural tendencies of clonal geckos with microhabitat use in a beach front forest. Through the ecological survey and behavioural tests, I detected the relationship between boldness (risk-taking tendency) and two microhabitat features: location of trees and perch height. This is the first report revealing that behavioural variation of clonal animals links to how each individual lives under nature. The observed pattern suggests that gradation of the artificial disturbance levels may link to boldness of resident geckos.

Key words

personality, boldness, clonal gecko, spacing pattern, microhabitat use, *Lepidodactylus lugubris*

Animal personality is defined as consistent behavioural differences among individuals, of which consistency is found through time, between situations, and across contexts (Sih et al. 2004; Réale et al. 2007; Carere and Maestripieri 2013). Across a broad range of taxonomic groups, some individuals are consistently more active, more aggressive, and bolder than other individuals (Gosling 2001; Bell et al. 2009; Garamszegi et al. 2012). Recently, behavioural ecologists have emphasized the importance of studying the adaptive significance of animal personality under natural conditions (Dingemanse and Réale 2005; Dingemanse and Wolf 2010). Indeed, several studies have found that personality types potentially influence various life histories such as growth, mortality, competition, and mating (Smith and Blumstein 2008; Biro and Stamps 2010; Briffa et al. 2015). In order to understand the relationships between behavioural tendencies and life-history traits, we should study how individuals with different personality types behave under nature.

Spacing and movement patterns of animals are ecologically significant aspects linking to both of individual fitness and population dynamics. At an individual level, movement and habitat-use influence how frequently individuals encounter competitors, mating partners, and predators (Englund 1997; Switzer 1997; Sims et al. 2006). At a population level, home range size and dispersal tendencies determine a distributional pattern, migration rate, and population size at a given area (Hawkes 2009; Brown and Crone 2016). In classical mathematic models, individuals within conspecifics were assumed as homogenous, but recent studies have revealed that individual difference of

spacing and movement patterns are associated with behavioural tendencies (Fraser et al. 2001; Cote et al. 2010; Nilsson et al. 2014; Spiegel et al. 2017). For example, social personality of the common lizard, *Zootoca vivipara*, influences dispersal tendencies of juveniles (Cote and Clobert 2007). Boldness score of the elk, *Cervus canadensis*, predicts migratory behaviour from urban to mountain areas during winter season (Found and St. Clair 2016). These findings suggest that animal personality is an important factor influencing individual movement within spatially diverse environment.

In spite of a lack of genetic variation, phenotypic variation of clonal organisms enables them to spread in spatially diverse environment. For example, branching coral, *Madracis mirabilis*, achieves local adaptation by morphological plasticity (Bruno and Edmunds 1997), and zooplankton, *Daphnia melanica*, resists predetors by phisiological plasticity (Scoville and Pfrender 2010). In addition to morphological and physiological traits, phenotypic variation is also found in behavioural trait as a personality among genetically identical animals (Schuett et al. 2011; Freund et al. 2013; Bierbach et al. 2017). Considering that animal personality potentially links with spatial ecological features, we can predict that personality of clonal animals allows them to spread in various environments. Although this prediction implies the importance of acquired personality variation, no empirical studies have shown behavioural tendencies associated with spatial ecological features of clonal animals.

The mourning gecko, *Lepidodactylus lugubris*, is an obligate parthenogenetic species, which reproduces genetically identical individuals without fertilization

(Moritz et al. 1993; Ineich 1999). Consistent behavioural differences among clonal individuals of *L. lugubris* have been detected in boldness and exploration, supporting the presence of personality (Sakai 2018). In addition, invasive populations of *L. lugubris* are widely expanding their distributional range across tropical and subtropical areas (Radtkey et al. 1995; Ineich 1999). Therefore, considering that genetically identical individuals have personality and live in diverse environments, *L. lugubris* is a suitable subject for investigating the significance of personality variation of clonal animals on spatial ecological features.

Here I investigated the relationship between behavioural tendencies and microhabitat use in an arboreal clonal gecko *L. lugubris*. First, I conducted a mark-recapture survey of free-living geckos in a small beachfront forest to investigate their spacing and movement patterns. Upon capture, I recorded microhabitat features: species of perch trees, location of the perch tree within the forest, and perch height. Based on encounter histories of recaptured individuals, I evaluated consistency of perch height and distance moved between capture events. In behavioural tests, I repeatedly assessed boldness and exploration of several individuals. Based on the ecological survey and behavioural tests, I examined possible association between microhabitat features and behavioural traits of each individual.

Methods

Field survey

133 Study site is a small beachfront forest (100 m length, 6–11 m width, 1068 m^2
134 area) that is located in the northern area of Okinawajima Island, Japan
135 (26°44′53.6" N, 128°10′46.7" E). The forest has two types of boundaries: (1) a
136 beach where people rarely and (2) a road where pedetorians and cars
137 frequently pass at night (Fig. 1). The forest consists of a coastal vegetation,
138 mainly sea hibiscus (*Hibiscus tiliaceus*; 8 m high), tropical almond (*Terminalia*
139 *catappa*; 11 m high), screw pine (*Pandanus odoratissimu*; 8 m high), and beach
140 cabbage (*Scaevola taccada*; 1 m high). To identify detail capture points of
141 geckos, I gave each tree a unique number and marked large branches with
142 yellow plastic tapes.
143 Populations of *L. lugubris* in Okinawajima Island are located in the
144 northernmost distributional area of its range (Ineich 1999; Yamashiro et al.
145 2000). These populations were introduced in the latter half of the twentieth
146 century by accidental human transportation, thus they are considered as
147 recently founded, non-native populations (Ota et al. 2004). Although this
148 species includes several clonal linages, only single clonal linage (a clone C
149 type) is distributed in the study area (Yamashiro et al. 2000; Sakai 2016). Each
150 clonal linage of *L. lugubris* can be distinguished by dorsal dot patterns (Ineich
151 1988; Ineich and Ota 1992), and molecular analyses support genetic uniformity
152 among individuals of the clone C type and validity of diagnosis based on the
153 dorsal dot patterns (Yamashiro et al. 2000; Murakami et al. 2015).
154 In total four field surveys were carried out over three years (September 2013,
155 June and September 2014, and September 2015). Each survey took

approximately 20 days to complete. I searched for geckos on trees by walking along the outside of the forest from sunset to midnight (approximately 4 hours) in three-day intervals. I collected geckos by hand and recorded three microhabitat features: tree species, location of trees within the forest, and perch height. Geckos were placed in small Ziploc bags (10 × 7 cm), which had been punctured with several small holes to allow air exchange, and they were immediately transported to an indoor field-laboratory close (< 1 km) to the beachfront forest. The day following capture, I measured their snout-vent length (SVL) to the nearest 0.1 mm using a digital calliper. Some geckos were then used for behavioural tests, and after measurement and behavioural tests, all geckos were marked with a toe clipping method and released at the original capture points. Except during the measurement process and behavioural tests, subjects were kept in the Ziploc bags and were placed in the room of the laboratory with natural light condition. I did not provide food and water to subjects during four days under the captive condition.

Behavioural tests

The dataset for behavioural trait is the same as the previous report that examined repeatability of boldness and exploration in *L. lugubris* (Sakai 2018). However, in this study, the dataset was used to investigate the relationship between gecko's behavioural tendencies and microhabitat use. The experiment was designed to evaluate behavioural repeatability and syndromes, thus I repeatedly conducted the same tests on three consecutive nights, each

179 including two separate assays for exploration and boldness. I started the first
180 test at the night following capture and conducted behavioural test once per night
181 for each individual. Within and across survey periods, I sometimes recaptured
182 individuals that had been previously assessed for boldness and exploration. In
183 these cases, the same three tests were carried out again, which was referred to
184 as a retest.
185 I conducted all experiments in the laboratory at night time (19:00–4:00) in
186 darkness because *L. lugubris* is a nocturnal gecko. Air temperature in the room
187 was controlled at 27.9 ± 1.2℃ (mean ± SD, N = 909 recordings) for all tests. I
188 recorded gecko's behaviour using a Sony digital video camera (HDR-XR550V)
189 set to night vision mode. The experimental arena was a plastic container (12 ×
190 20 × 12 cm), in which grid lines (4 × 4 cm) had been drawn on the walls and
191 floor. A refuge made of a card board (5 × 5 × 1.5 cm) was set at the centre of
192 the arena's floor. I cleaned the inside of each container with wet tissue paper
193 after each trial. To minimize observer bias, blinded methods were used when all
194 behavioural data were recorded and analysed.
195
196 Novel environment assay for exploration
197 For acclimation, subjects were set in the dark experimental room for at least 30
198 min before tests. I turned on room light and gently introduced a gecko from the
199 Ziploc bag into the experimental arena without grasping. I immediately turned
200 off light and left the room, then I recorded gecko's behaviour for the next 40 min
201 with the video camera. Exploration is generally defined as the reaction to a

novel environment or situation (Réale et al. 2007; Carter et al. 2013). Thus, in this assay, the number of grid lines that the gecko crossed was counted as the indicator of exploration because the experimental arena was a potentially novel environment for wild geckos. Given that geckos sometime hid within the refuge or walked on a celling that has no grid lines, I firstly calculated the total exploring duration when geckos were out of the refuge and off the celling. Then, I divided the number of grid lines that the gecko crossed by the total exploring duration (min). These calculations allow us to evaluate a relative exploration per 1 min of each subject with different exploring duration.

Threat stimulus assay for boldness

After the novel environment assay, I immediately entered the experimental room and turned on light. I proceeded to chase the gecko around the arena by hand to induce it hide into the refuge. It should be noted that chasing duration ranged from 10 to 60 sec depending on trials, but I standardized a situation that geckos voluntarily run into the refuge. I immediately turned off the room light and left the room, then I recorded gecko's behaviour for a further 20 min. Boldness is generally defined as the individual's risk avoidance tendency (Wilson et al. 1994; Réale et al. 2007). Thus, in this assay, total hiding duration within the refuge was used as the negative indicator of boldness because chased stimulus was a threat experience that geckos would perceive as being similar to a predatory attack. Therefore, longer hiding duration represents a risk-averse

224 tendency (shy), whereas shorter hiding duration represents a risk-prone
225 tendency (bold).
226
227 Data analysis
228 Inhabiting site and movement pattern
229 I recorded all 626 capture locations at trees within the forest, and 396 out of
230 them at branches within each tree. The number of capture events of each
231 individual ranged from 1 to 10 times (*N* = 369 individuals, mean ± SD = 1.70 ±
232 1.17 times). For 131 individuals of which precise capture points had been
233 recorded at least twice, I assessed consistency of perch height. I analysed
234 consistency by intraclass correlation coefficient (ICC); it represents within-
235 individual variance compared to total variance (sum of within and among
236 individual variance) (Nakagawa and Schielzeth 2010). ICC ranges from 0 to 1,
237 and it is close to 1 when geckos exhibited a small range of perch height within
238 individual compared to a wide range of perch height among individuals.
239 Restricted maximum likelihood based linear mixed-effects model was selected
240 for the calculation using the R package "*rptR*" (Nakagawa and Schielzeth 2010).
241 Perch height was set as a response variable, and individual identity and survey
242 period (Sep 2013, Jun 2014, Sep 2014, and Sep 2015) were set as random
243 factors.
244 To evaluate a movement pattern, I examined a concordance of capture points
245 of individuals. In addition, I calculated the distance moved between capture
246 points of the same individuals. In short distance (< 10 m), I directly measured

distance between captured points by a tape measure, which reflects 3-dimentional distance. In long distance (> 10 m), I calculated distance between capture points based on the map of the field site, which reflects 2-dimentional distance. Time interval between captures should be taken into account because longer duration allows animals to travel longer distance. In the present survey, recapture events were divided into two time scales; within each survey period (*N* = 207 recordings, mean ± SD = 6.9 ± 4.4 days, range 4 to 23) and across survey periods (*N* = 50 recordings, 278.5 ± 129.7 days, range 71 to 477). Thus, I analysed movement patterns in short (within survey period) and long (across survey periods) time scales, separately.

Behavioural tendency and microhabitat features

I conducted behavioural tests for 44 juveniles (SVL: 24.1 ± 3.4 mm, mean ± SD) and 41 adults (SVL: 43.1 ± 2.7 mm), and obtained 112 observations including 37 retests. Only geckos captured from tropical almond trees (42 observations for juveniles and 56 observations for adults) were used for the following analyses because most geckos were collected from this tree (see Results). Mean value of three tests was used as individual boldness and exploration scores. To assess the relationship between behavioural tendencies and microhabitat features, I conducted two step analyses for a general pattern and group compositions.

For a general pattern, I applied univariate linear mixed-effect models for boldness and exploration scores. Two microhabitat features with different spatial

scale were set as fixed factors: forest side and perch height. Forest side is binominal parameter based on location of trees within the forest (beach side or road side, Fig.1), and perch height is continuous parameter (meters). Individual identity and survey period were set as random factors. R package "*lmerTest*" was used for these analyses, and restricted most likelihood t-test using Satterthwaite approximations for degrees of freedom was used to determine the significance of fixed factors (see details in Kuznetsova et al. 2015).

For group compositions, I applied further analyses to only boldness because I detected the general pattern only in this trait (see Results). First, I changed continuous variables to categorical variables. I divided geckos into two types based on boldness score, which ranged from 0 to 20 min in hiding duration (bold: under 10 min; shy: over 10 min). I also divided perch height into two classes (low branches: under 2.8 m; high branches: over 2.8 m) because perch height in tropical almond trees ranged from 1.6 to 4.5 m. Differences between the two microhabitat classes in the number of individuals categorized in two boldness types were analysed using a Fisher's exact probability test for 2×2 tables. So I applied a Fisher's exact probability test for the forest side (beach side/road side × shy/bold) and perch height (low branch/high branch × shy/bold), respectively. Retest data was excluded from these analyses to maintain independence of data, therefore only one dataset was used for each individual. All statistical calculations were conducted using the software R ver. 3.4.1.

293 RESULTS

294 Inhabiting sites and movement pattern

295 Geckos showed a clumped distribution in specific trees within the forest. I
296 captured 577 geckos (92.2 % of all captures) in tropical almond, 36 geckos
297 (5.8 %) in sea hibiscus, and the remaining 13 geckos (2.1 %) in other species of
298 plants, respectively. Both juveniles and adults were collected from low to high
299 branches, and perch height was not biased by body size of geckos (Fig. 2).
300 Geckos exhibited consistency in perch height within individuals; some geckos
301 were repeatedly collected from high branches, whereas other geckos were
302 repeatedly collected from low branches (ICC = 0.62, 95%CI = 0.46–0.74, $P <$
303 0.001).

304 Encounter histories of geckos revealed a high level of site fidelity and a
305 restricted movement pattern within a small area. Two hundreds six out of 207
306 recaptures (99.5 %) in short time scale and 43 out of 50 recaptures (86.0 %) in
307 long time scale were occurred from the same trees where each gecko had been
308 previously collected. Distance between capture points were 0.52 ± 1.08 m
309 (mean ± SD, N = 131 recordings) in short time scale and 5.01 ± 9.77 m (N = 37
310 recordings) in long time scale. However, these mean values were highly skewed
311 by leptokurtic distribution; most individuals did not move longer than 3 m and a
312 few individuals travelled longer distance over 10 m (Fig. 3).

313

314 Behavioural tendency and microhabitat use

315 In the general pattern, exploration score was not related to microhabitat

316 features (LMM: forest side, t = 0.93, P = 0.356; perch height, t = -0.15, P =
317 0.884). On the other hand, boldness score was significantly related to
318 microhabitat features; geckos from beach side were bolder than those from
319 road side (LMM: t = -2.18, P = 0.033), and their boldness increased with perch
320 height (LMM: t = -3.19, P = 0.002). Relatively bold geckos were collected from
321 both sides, while relatively shy geckos were collected from only road side trees
322 (Fisher's exact probability test: P = 0.002) (Fig. 4). Similarly, relatively bold
323 geckos were collected from low to high branches, while relatively shy geckos
324 were collected from only low branches (Fisher's exact probability test: P =
325 0.028) (Fig. 5).
326
327 Discussion
328 The mark-recapture survey revealed the following spatial ecological features of
329 *L. lugubris* living in a small beachfront forest. First, geckos were clumped in the
330 specific tree species. Second, geckos exhibited consistency of perch height
331 within individuals. Third, geckos showed a high level of site fidelity at the same
332 tree and moved short distance over long time scale. In addition, I detected that
333 boldness score of resident geckos was related to tree location within the forest
334 and perch height. Considering that the focal geckos exhibited consistency of
335 perch height and a high level of site fidelity over long time, the present results
336 strongly suggest the relationship between risk-taking tendencies and
337 microhabitat use of clonal geckos.
338

Spatial ecological features

Clumped distribution in the specific trees within a forest suggests that *L. lugubris* has a preference of inhabiting trees. The availability of nectar may attract geckos. The mourning gecko is basically insectivorous, but it occasionally licks nectar of plants (Perry and Ritter 1999). The tropical almond has extrafloral nectar glands on leaves (Aguirre et al. 2013), and mourning geckos in the focal population frequently lick this nectar (Sakai 2015). Similar behaviour has been reported from other gecko species. For example, in the Pacific gecko *Hoplodactylus pacifics*, as many as 50 individuals aggregated at a flowering pohutukawa tree (*Metrisoderos* spp.) feeding on nectars together (Whitaker 1968). Therefore, the availability of nectars may generate locally dense distribution of *L. lugubris* in the study site.

High site fidelity of *L. lugubris* supports the previous reports in which its home range size usually does not exceed an area of 1.8–2.4 m in diameter (Hunsaker and Breese 1967; Lianna and Lazell 1987). Home range size of lizards is generally determined by foraging mode, dietary niche, and mating role in reproduction (Stamps 1977; Perry and Theodore 2002). Sit-and-wait foraging mode, insectivorous but sometime nectivory diet, and parthenogenetic reproduction (without seeking mating partners) may not require large home range. However, it is not clear whether observed spacing and movement patterns are characteristics unique to *L. lugubris* because few information about spatial ecology has been accumulated in arboreal gecko species (Stamps 1977; Perry and Theodore 2002). For further discussion, investigation of spatial

362 ecological features in various gecko species are required.

363

364 Boldness associated with microhabitat

365 The present results suggest that environmental gradient within the forest
366 contributes to boldness score of resident geckos. Boldness is defined as a risk
367 prone tendency and relates to avoidance from predators in the wild (Wilson et
368 al. 1994; Réale et al. 2007). For example, predatory pressure would influence
369 boldness score of the three spined stickleback, *Gasterosteus aculeatus*, and the
370 guppy, *Poecilia reticulata* (Bell and Sih 2007; Harris et al. 2010). However, it is
371 unlikely that predatory pressure is gradient within the study site because forest
372 width and range of perch height are very narrow (c.f. width: 6–11 m, perch
373 height: 0.8–5 m). One reasonable environmental gradient within the study site is
374 artificial disturbance. Relationship between artificial disturbance and boldness
375 has been reported in the burrowing owl, *Athene cunicularia* (Carrete and Tella
376 2010), the yellow-bellied marmot, *Marmota flaviventri* (Petelle et al. 2013), and
377 the dunnock, *Prunella modularis* (Holtmann et al. 2017). In the present study
378 site, it is possible that geckos living in low branches of road side trees have
379 much experienced artificial disturbance than those living in high branches or
380 beach side trees.

381 Then, the question turns to what mechanisms generate the observed
382 association between boldness and microhabitat use. One possible clue is the
383 developmental process of personality forming. A previous study showed that
384 small size juveniles of *L. lugubris* are all bold and that appearance of shy

individuals throughout ontogeny generated personality variation within the focal population (Sakai 2018). In addition, the high level of site fidelity is likely to be applied throughout life stages; some individuals were collected from the same trees when they were juvenile and adult stages. Therefore, geckos living in the frequently disturbed area may gradually change to be shy, whereas geckos living in the disturb less area may maintain their boldness.

Another possible mechanism is microhabitat choice associated with boldness. In general, shy individuals are more sensitive to environmental conditions compared to bold individuals (Wolf et al. 2008; Quinn et al. 2012), and such sensitivity induces differences of choosiness; some animals tend to select a specific environment but other animals do not (Pruitt et al. 2011). This possibility would fit to the present results; relatively bold geckos were collected from any microhabitats, whereas relatively shy geckos were collected from a restricted microhabitat within the forest. In the focal population, approximately 15 % of individuals migrated to other trees in long time scale. Thus, it is possible that shy individuals may select a specific microhabitat because of their sensitivity to environment but bold individuals may stay the same site regardless of environmental conditions. In this scenario, however, it is still unknown why shy individuals prefer low branches on road side trees.

Conclusions

The present study revealed that boldness of clonal geckos is associated with microhabitat features: forest side and perch height. This is the first report

408 revealing that behavioural variation in clonal animals links to their microhabitats
409 use. Acquired personality of clonal animals (not based on genetic variation) may
410 allow them to use a wide range of microhabitats that are not occupied by other
411 individuals. If individual specialization in boldness allows clonal animals to adapt
412 to local environments, generating personality variation may lead to a successful
413 invasion of *L. lugubris* to diverse environments. In the present study, however, it
414 is difficult to conclude what environmental factors affect the boldness score of
415 resident animals and what mechanisms generate the observed pattern. Further
416 investigations are required to clarify the developmental process of personality
417 forming on each microhabitat and habitat choice associated with boldness.
418

Compliance with ethical standards

Funding: This research was conducted without any funding support.

Conflict of interest: The author declare no conflicts of interest associated with this manuscript.

Ethical approval: The present study was carried out in compliance with the guideline of the Animal Care and Use Committee of Kyoto University. The field survey was conducted under the permission of the Department of Agriculture, Forestry and Fisheries of Okinawa Prefecture. Field survey and experimental procedures were designed to minimize stress and harm in subjects.

Data availability: The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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599

600 Figure legends

601

602 **Fig. 1** Map of the study site. Light green stripes: sea hibiscus, dark green
603 ellipse: tropical almond, green dots: screw pine and beach cabbage. Based on
604 location within the forest, tropical almond trees (TA) were categorized as road
605 side (#2, #3, #4) and beach side (#1, #5, #6).

606

607 **Fig. 2** Perch height of mourning geckos from a beachfront forest.
608 Developmental stage was determined based on SVL (snout-vent length) of
609 sexually maturation size (Sakai 2016).

610

611 **Fig. 3** Distance between the recapture and the preceeding capture points of
612 mouning geckos. Short intervals represent recaptures within each survey period
613 (4-23 days) and long intervals represent recaptures across survey periods (71-
614 477 days). Histogram is made by log-transformed value, but x-axis is described
615 by the original scale (meter).

616

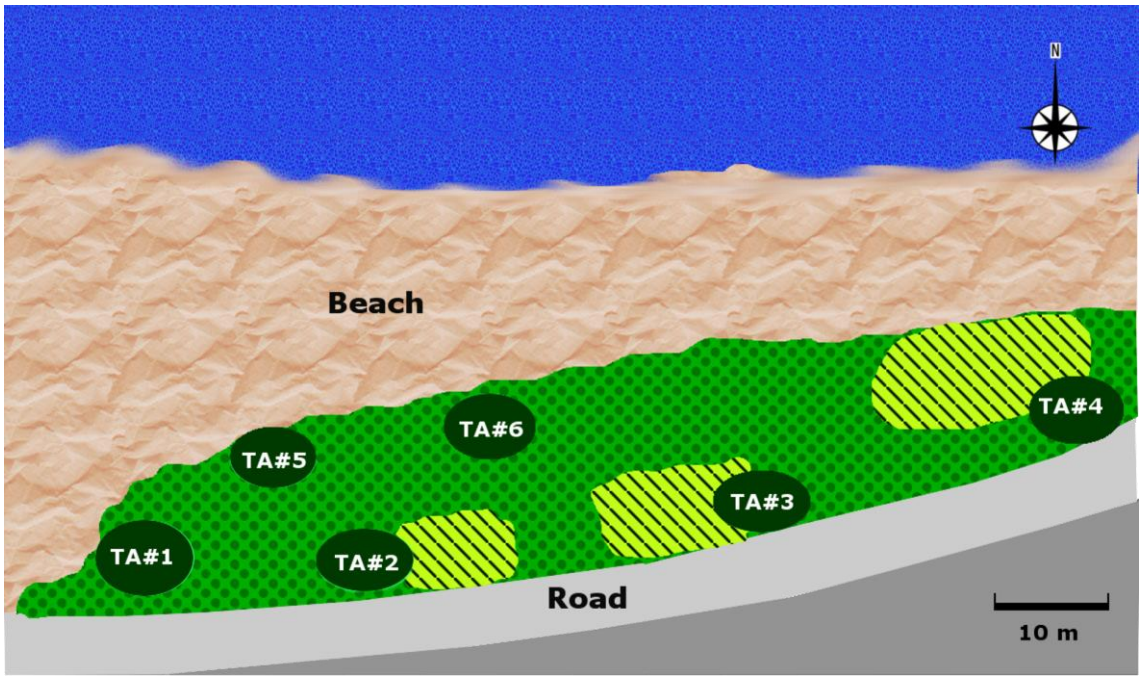
617 **Fig. 4** Comparison of boldness score of resident geckos between road side and
618 beach side tropical almond trees.

619

620 **Fig. 5** Boldness score of resident geckos across perch height of tropical almond
621 trees.
622

623 FIGURES

624 Fig.1



625

626

627 Fig.2

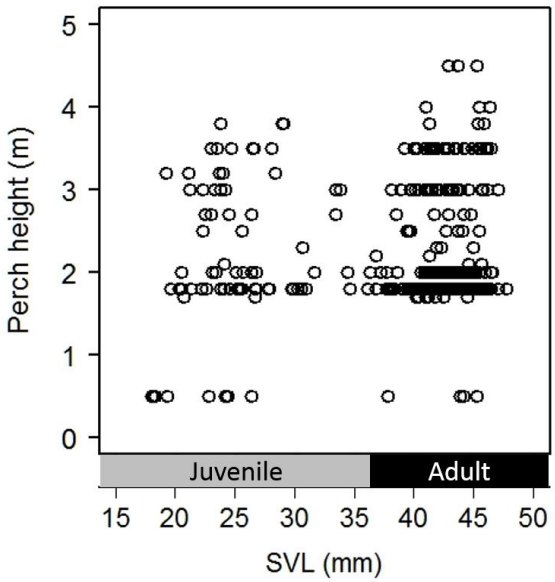
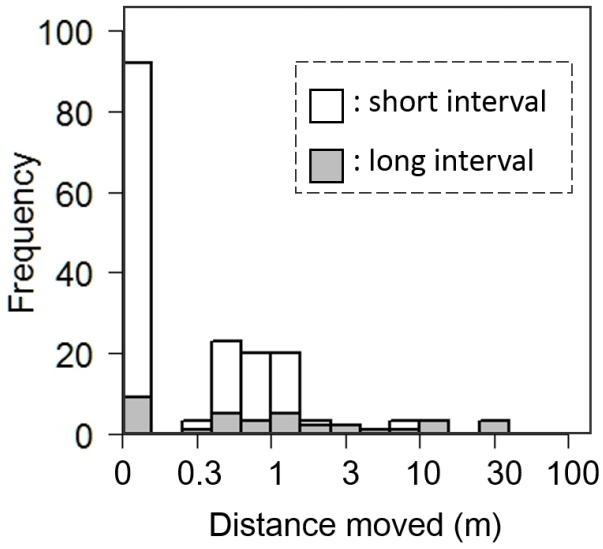


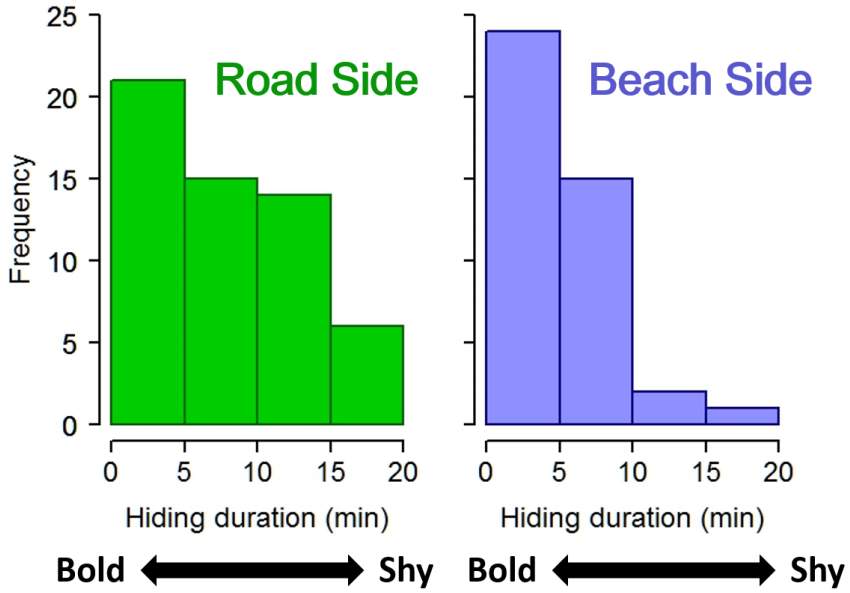
Fig.3



628

629

630 Fig.4



631

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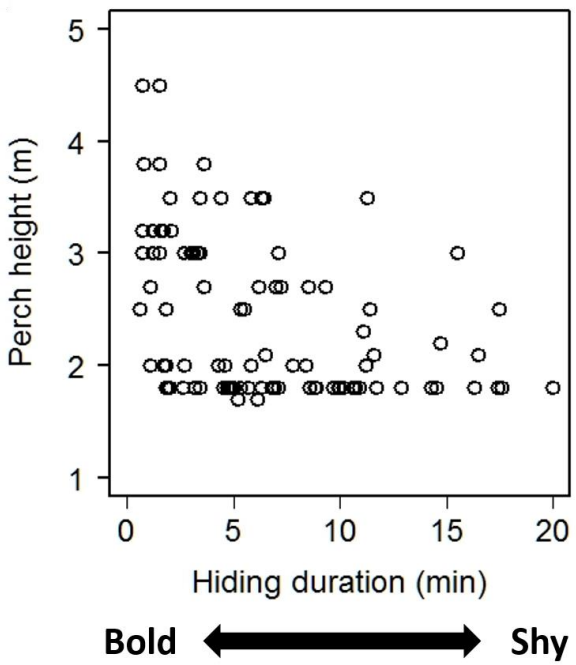
633 Fig.5



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TITLE PAGE

Does different energy intake gradually promote personality variation throughout the life stage in a clonal gecko species?

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ABSTRACT

With accumulating evidence of both the presence and absence of consistent behavioural differences within conspecifics, various hypotheses have been proposed to explain mechanisms of personality formation. Several models predict that interactions between state and behaviour gradually generate, or sometime diminish, individual variation with ontogeny. However, these models should be empirically tested with a simple study system. Here, I investigated whether state differences via energy intake promote consistent behavioural differences among clonal geckos. I controlled food supply of two groups (high or low energy intake) and evaluated their life-history traits and behavioural traits throughout the development. Geckos at several age classes exhibited consistent differences in risk-boldness, foraging-boldness, and activity. Differences between the two groups were found in growth rate and reproduction but not in foraging-boldness and activity. The exception was risk-boldness; high energy group maintained to be bold throughout the development, whereas low energy group changed to be shy at early stages and returned to be bold at later stages. These results imply that differences in energy intake do not gradually change behavioural traits with ontogeny, suggesting that the predictions of previous simple models are not supported. Considering that predation risk and food availability change with the development of geckos, the state-dependent safety model could explain divergence and convergence of risk-boldness at different life stages. The present study highlights the importance of life stage specific effect of state on behaviour for personality formation.

KEY WORDS

personality formation, development, pace-of-life syndrome, asset protection, state-behaviour feedback loop, energy intake

INTRODUCTION

Over the past decades, accumulating empirical studies have reported that individuals within conspecifics exhibit consistent behavioural differences and correlated behavioural traits (Koolhaas et al. 1999; Gosling 2001; Réale et al. 2007; Bell et al. 2009). These behavioural characteristics are described as animal personality (e.g., boldness in risk-taking tendency, aggression in agonistic interaction, and exploration to novel situation), and correlated structure between behavioural traits is described as behavioural syndrome (Sih et al. 2004; Garamszegi et al. 2012; Carere and Maestripieri 2013). However, some studies also have found that, even within the same species, the presence of animal personality and behavioural syndrome depend on populations and life stages. For example, some populations in sticklebacks show a correlation between boldness and aggression, but other populations lack behavioural syndromes (Bell 2005; Dingemanse et al. 2007). In other cases, behavioural repeatability is observed in a specific life stage but not in other life stages (Petelle et al. 2013; Favati et al. 2016). By making various hypotheses, researchers have challenged to understand why and how consistent behavioural differences are generated, maintained, and sometime disappeared (Dall et al. 2004; Wolf et al. 2007; Wolf and McNamara 2012; Dingemanse and Wolf 2013).

One of these hypotheses, the feedback loop hypothesis, is a comprehensive idea that can be applied to several intrinsic state and behaviour of animals (Sih et al. 2015; Stamps and Biro 2016). This hypothesis assumes that individuals

change behaviour based on their state variables and that behavioural tendencies also change individuals' state. Especially, intrinsic energy state is considered to be highly associated with boldness, activity, aggressiveness, and foraging proactivity (Luttbeg and Sih 2010; Sih et al. 2015; but see Niemelä and Dingemanse 2018). Feedback process between state and behaviour promotes or reduces consistent differences among individuals; a positive feedback loop gradually increases variation, whereas a negative feedback loop gradually decreases variation through time (Sih et al. 2015; Stamps and Biro 2016). One advantageous point of this hypothesis is that it can predict developmental trajectories of behavioural traits, thus it helps to understand mechanisms of personality formation. However, even with the same combination of state variable and behavioural trait, the feedback loop leads to opposite predictions of behavioural trajectories depending on the underlying conceptual mechanisms. Pace-of-life syndrome and asset protection models provide good examples for such contradicting mechanisms with energy state.

The concept of pace-of-life syndrome (POLS) explains integration of physiological, behavioural, and life-history traits as a slow-fast continuum (Réale et al. 2010). The classic framework of pace-of-life was originated from interspecific variation (Gaillard et al. 1989; Bielby et al. 2007), but recently it is extended to intraspecific variation (Réale et al. 2010; Mathot and Frankenhuis 2018). A physiological cause of pace-of-life is basal metabolism, and trade-offs between productivity and mortality bring about individual variation (Biro and Stamps 2008, 2010; Mathot and Dall 2013). Fast life strategy attaches weight to

high productivity at the expense of safety, thus fast life animals (e.g., high metabolic rate, high growth rate, and early reproduction) show risk taking propensity and high activity. On the other hand, slow life strategy attaches weight to safety at the expense of low productivity, thus slow life animals (e.g., low metabolic rate, low growth rate, and delayed reproduction) show risk avoidance propensity and low activity. When this concept is applied to the feedback loop, initial differences of energy state would induce differences of basal metabolic rate among individuals. Individuals with high basal metabolic rate grow faster and behave bolder to acquire much energy intake, whereas individuals with low basal metabolic rate grow slower and behave shyer because low energy intake is enough to maintain a slow life (Sih et al. 2015). Overall, it can be predicted that individual differences of pace-of-life and associated behavioural traits gradually increase with development.

The other concept, asset protection model, assumes that animals plastically change foraging effort and activity based on their energy budgets (Clark 1994). In a case that higher foraging effort increases both food acquirement and predation risk, individuals with a high energy budget exhibit shy tendency and low activity to avoid predation risk, whereas individuals with a low energy budget exhibit risk-prone tendency and high activity to avoid starvation risk. Therefore, individual differences of energy budgets cause individual variation of risk taking tendency, foraging proactivity, and activity (Clark 1994). When this concept is applied to the feedback loop, it can be predicted that initial differences of energy state and associated behavioural traits eventually

115 converge to the same levels (Luttbeg and Sih 2010). Thus, this negative
116 feedback process gradually decreases differences of state and behavioural
117 traits among individuals.

118 Although the POLS and asset protection models predict opposite effect of
119 energy intake on behavioural traits, these concepts are not mutually exclusive.
120 A relative importance of these models would depend on biological backgrounds
121 such as ecological, physiological, and taxonomic constraints (Luttbeg and Sih
122 2010; Réale et al. 2010; Sih et al. 2015). For example, a recent meta-analysis
123 found that the POLS model is supported in invertebrates than vertebrates
124 (Royauté et al. 2018). Nonetheless, some vertebrate animals such as domestic
125 dogs (*Canis familiaris*) exhibit a strong association of several traits as the POLS
126 model predicts (Careau et al. 2010). To find out what factors determine
127 dominant mechanisms, it is required to verify these models in various animal
128 species. In addition, studies with simple experimental systems are desired to
129 unravel the entangled relationships among state variables, life-history traits, and
130 behavioural traits in each model.

131 Clonal animals are suitable subjects for a simple and powerful approach to
132 reveal personality formation because we can exclude the effects of genetic
133 variation on phenotypic variation (Stamps and Groothuis 2010; Freund et al.
134 2013; Bierbach et al. 2017). The mourning gecko (*Lepidodactylus lugubris*) is
135 an obligate parthenogenetic species, which reproduces genetically uniform
136 individuals without fertilization (Ineich 1999). Previous studies have shown that
137 free-living clonal geckos exhibit consistent differences in risk taking and

exploratory behaviours (Sakai 2018). In addition, behavioural variation of clonal geckos increases with developmental stage, suggesting that the non-genetic factors generate personality variation among clonal individuals (Sakai 2018). Given that the mourning gecko is easily maintained in a laboratory condition, it is the suitable subject for examining the effect of state variables on behaviours from the developmental aspect.

The present study aimed to understand the cause and mechanism of personality formation in a clonal gecko species throughout their development. First, I aimed to investigate whether state variables via energy intake affect life-history traits and behavioural traits. Second, I aimed to examine a relative applicability of the POLS and asset protection models to mourning geckos. Third, I aimed to investigate whether the changing process of behaviour is gradual with the development as the feedback loop hypothesis predicts. I designed the experiment so that state variable is intentionally manipulated by a food supply but is not changed by subjects' behavioural tendency (Fig.1a, c). To induce consistent differences of energy intake between individuals, I divided laboratory hatched *L. lugubris* into two groups and provided different amounts of food over 15 months. Because of difficulty to assess intrinsic state variables, I evaluated their life-history traits (growth rate and reproduction), which are considered to reflect subjects' energy state. I also measured three behavioural traits (risk- boldness, foraging-boldness, and activity) every three months. If the POLS model is applicable, it is predicted that individuals with a high energy intake tend to be bolder and highly active than those with a low energy intake

(Fig. 1b). Alternatively, if the asset protection model is applicable, it is predicted that individuals with a high energy intake change to be shyer and less active than those with a low energy intake (Fig. 1d).

METHODS

Subjects

In order to use naïve individuals, I prepared F_1 and F_2 generations of *L. lugubris* that were hatched in the laboratory. The origins of these geckos were populations in Ryukyu Archipelago, Japan. These populations were introduced during the latter half of the twentieth century and are assumed as recently founded populations (Ota et al. 2004). *Lepidodactylus lugubris* includes several clonal linages that can be distinguished by dorsal dot patterns (Ineich 1988; Ineich and Ota 1992). Only single clonal linage (clone C type) is distributed in the study area, and molecular analyses support validity of the diagnosis based on the dorsal dot patterns and genetic uniformity among individuals of clone C type (Yamashiro et al. 2000; Murakami et al. 2015). I captured 14 and 20 gravid females from Ishigaki-jima Island (May 2016) and Okinawa-jima Island (June 2016), respectively. In addition, I used four gravid F_1 individuals of which mothers were captured from Okinawa-jima Island (June 2013). These gravid females were individually maintained in a small container (175×105×105 mm) under a constant room temperature (25℃) with a 12-h light and 12-h dark cycle. I sprayed water mist and provided five crickets (*Gryllus bimaculatus*) dusted

184 with calcium powder twice every week.

185 A total of 53 eggs were collected from 38 mothers during four months (9th
186 May to 25th August, 2016), and these eggs were separately incubated under a
187 constant room temperature (25℃). Forty-four eggs were hatched within three
188 months, and the remaining nine eggs were not hatched over five months since
189 the oviposition. Upon hatching, I measured subject's snout-vent length (SVL) to
190 the nearest 0.1 mm with a digital calliper and body mass to the nearest 1 mg
191 with a digital balance. Subjects were divided into two groups without
192 morphological bias (Welch's two sample t-test; SVL: t = 0.356, df = 41.2, p =
193 0.723; Body mass: t = 0.402, df = 40.9, p = 0.690) and body condition bias
194 (Body condition index: t = 0.536, df = 37.5, p = 0.595), of which score was
195 assessed from residuals of body size/body mass regression model (Jakob et al.
196 1996). In addition, given that clutch size of *L. lugubris* is typically two, siblings
197 were divided into different groups.

198

199 *Husbandry and feeding protocol*

200 Geckos were individually maintained in a small container (175×105×105 mm)
201 under a constant room temperature (25℃) with a 12-h light and 12-h dark cycle.
202 The floor of the container was covered with a paper towel, and the outside walls
203 were surrounded with card boards to prevent visual interactions between
204 individuals. A cylindrical water bowl (diameter 28 mm, depth 15 mm) and a
205 cardboard refuge (50×50×15 mm) were set at one of the corners. I replaced the
206 container, the floor paper, and the refuge to new ones every month. The

207 containers were placed on shelves, and the position of each container was
208 haphazardly moved every week to minimize the position bias. When geckos
209 reached sexually matured size (SVL > 36.8 mm, Sakai 2016), I carefully
210 checked the gravid condition and recorded age at the first oviposition.
211 To induce consistent differences of energy intake between the two groups, I
212 provided different amounts of crickets (*Gryllus bimaculatus*) to each group
213 throughout the life stage. One group (high energy group) consistently consumed
214 a larger amount of crickets than the other group (low energy group) until
215 subjects reached the age of 12 months. Thereafter, I continuously provided the
216 same amount of crickets to both groups until their first reproduction. Because
217 energy requirement increases with growth, I raised the amount of food supply
218 every three months (Table 1). Given that gape size of geckos restricts available
219 prey size, I gradually changed cricket size to larger one (from 1 mm to 6 mm in
220 body length) as geckos grew. I provided crickets and sprayed water mist twice
221 every week. Geckos usually consumed all crickets within a day, and no leftovers
222 were observed in the next feeding day. Therefore, the present feeding protocol
223 strictly controlled energy intake of subjects.
224
225 *Behavioural tests*
226 I conducted a series of three types of assays (threat, food, and activity) every
227 three months of subject's age (0.2, 3, 6, 9, 12, and 15 months). This time scale
228 covers a developmental trajectory of *L. lugubris* from hatching to sexual
229 maturation (See Fig. 5 in Results). Note that I started the feeding protocol after

the first behavioural tests, thus there was no differences of energy intake between the two groups when subjects were tested at the age of 0.2 month. In addition, I repeatedly conducted each assay in consecutive three trials (1st, 2nd, and 3rd) at each age. This experimental design allowed to evaluate both consistent behavioural differences at each age and behavioural stability over the extended period of the ontogeny. A detailed schedule of behavioural tests was described in Figure 2. One day before the tests, I replaced the container to new one and provided crickets to standardize subject's hunger condition. Then, I conducted threat and food assays from day 2 to day 4 and activity assay from day 5 to day 7. The day following, I measured gecko's morphological traits (SVL and body mass).

Threat assay

The experiments were conducted in a dark room at night and were recorded by a digital video camera (Sony FDR-AX55) set to night vision mode because *L. lugubris* is nocturnal. Air temperature in the room was controlled at 25℃ for all tests. The experimental arena was a plastic container (200×120×120 mm), and a cardboard refuge (50×50×15 mm) was set at one of the corners (Fig. 3a). For acclimation, I placed the container of geckos in the dark room for at least 30-min, then I chased a subject by hand for 20 sec within the container and captured it by a Ziploc bag (100×70 mm). This chased stimulus was considered a threat experience that geckos would perceive as being similar to a predatory attack. I immediately introduced a subject from the Ziploc bag into the

253 experimental arena and induced it in the refuge. Then I turned off the room light
254 and recorded gecko's behaviour for the subsequent 30-min by the video camera
255 without observers. By analysing the video records, I measured latency to
256 emerge from the refuge (min), total hiding duration (min), and the number of the
257 refuge uses (times).
258
259 Food assay
260 After the threat assay, the subject was remained in the experimental arena and
261 was placed in the dark room for at least 30-min. Thereafter, I tuned on the room
262 light and waited until the subject voluntarily hid in the refuge. This situation
263 standardized the gecko's position within the arena. In most cases, the subject
264 immediately hid in the refuge when I entered the experimental room. Then I put
265 a transparent cup (diameter 65 mm, depth 35 mm) that contained 10 live
266 crickets in front of the refuge (Fig. 3b). After setting the cup, I left the room and
267 recorded gecko's behaviour for the subsequent 30-min by the video camera.
268 The subject was able to recognize crickets from the inside of the refuge but was
269 not able to feed on them. I defined the nearby area around the cup as a food
270 area (80×80 mm) and measured four variables: latency to approach the food
271 area (min), total duration on the food area (min), the number of times the gecko
272 entered the food area (times), and the number of strikes to the cup (times). After
273 the food assay, I returned the subject to the original container.
274
275 Activity assay

This assay was conducted in the keeping room under a constant room temperature (25℃) in a 12-h light and 12-h dark cycle. Activity was evaluated by a sensor system that can be attached on the outside of the container. I implanted infrared laser sensors (EE SPW-321, Omron, Kyoto, Japan) in white plastic boards and surrounded the container by these boards. I set three laser beams above 5 mm from the surface of the floor and walls (Fig. 3c). I gently removed objects (water bowl, refuge, and floor sheet) from the container and attached the sensor system on the outside of the container. I started assays between 13:00–17:00, which is the mid-period during the light phase (9:00–21:00), and continued the measurement over 72-hours. I summed the number of laser beam crosses every 24-hours as a day activity score. Therefore, I obtained three days activity scores for each gecko at each age (1st: 0–24 h, 2nd: 24–48 h, and 3rd: 48–72 h).

Data analysis

All statistical calculations were conducted using the software R ver. 3.4.1 (R Core Team 2017), and the significance level was set as $\alpha = 0.05$. I successfully obtained data of 24 individuals throughout 15 months (high energy group: N = 13, low energy group: N = 11) and one individual of high energy group until the age of 6 months, thus I used data set of 441 recordings from 25 individuals in the subsequent analyses.

Behavioural traits

First, I reduced the measured behavioural variables to a smaller number of synthetic variables using principal components analyses (PCA) for the threat assay and food assay separately. To simplify the analyses and interpretations, I chose only the first principal component from each assay. In the threat assay, the first principal component explained a total of 62% of the variance with high negative loadings on total hiding duration and latency to appearance (Table 2). This principal component represents risk-prone tendency as boldness in previous studies (Wilson et al. 1994; Réale et al. 2007), thus I used this score as an indicator of “risk-boldness”. In the food assay, the first principal component explained a total of 51% of the variance with a high negative loading on latency to approach the food area, and positive loadings on total duration in the food area and the number of entering the food area (Table 2). This principal component represents proactive-reactive tendency on feeding situation (Réale et al. 2007; Short and Petren 2008), thus I used this score as an indicator of “foraging-boldness”.

In the subsequent analyses, I focused on the key features of animal personality and its structure, thus I analysed (1) behavioural repeatability and (2) behavioural syndrome in all age classes. I also focused on whether behavioural tendency was maintained over the extended period of ontogeny as (3) behavioural stability across ages.

(1) Behavioural repeatability

To assess consistent behavioural differences among individuals, I evaluated repeatability in consecutive three trials using intraclass correlation coefficient (ICC) (Nakagawa and Schielzeth 2010; Dingemanse and Dochtermann 2013). I calculated ICC repeatability for each of the three behavioural traits in each age class. Fitting in linear mixed effect models (LMMs) using R package "*rptR*" (Nakagawa and Schielzeth 2010), I set the energy intake (high or low energy) and the number of trials (1st, 2nd, and 3rd trials) as fixed factors. I also set individual identity as random intercepts and random slopes. Based on bootstrap trials (*N* =1,000 times), I calculated the estimate and 95% confidence intervals of ICC repeatability. In addition, a log-likelihood ratio test was used to determine the significance of random factors between models with and without individual identity.

(2) Behavioural syndrome

To assess behavioural syndrome structure (correlation between behavioural traits in different context), I used Spearman correlation coefficient between three combinations of behavioural traits. Mean value of three trials was used as individual score of risk-boldness, foraging-boldness, and activity.

(3) Behavioural stability across age

To assess whether the rank order of behavioural tendency was maintained over the extended period of ontogeny, I used Spearman correlation coefficient in each behavioural trait of the same individuals across ages. In this analysis, a

strong positive correlation indicates a stable behavioural tendency over the ontogeny. Mean value of three trials was used as individual score for each behavioural trait. I calculated correlations between two adjacent age classes for all behavioural traits.

Effect of food control

To examine whether different amounts of food induced differences of life-history traits, I compared growth rate and age at the first oviposition between the high and low energy groups. Growth rate was calculated by the following formula (Andrews 1982):

$$\text{Growth rate (\%)} = 100(\ln W_{i+1} - \ln W_i) / (t_{i+1} - t_i)$$

where “ln” is the natural logarithm, “W” is the body mass (mg) when the subject’s age of “t_i” (month). I assessed growth rates for five time periods across the age 0.2–3, 3–6, 6–9, 9–12, and 12–15 months. Welch’s two sample t-test was used for comparing these life-history traits between the two groups.

In addition, to assess differences of behavioural traits between the low and high energy groups, I applied LMMs for three behavioural traits at each age class using the R package “*lmerTest*”. I set the energy intake (high or low energy) and the number of trials (1st, 2nd, and 3rd trials) as fixed factors. I also set individual identity as random intercepts and random slopes. The significance of fixed factors were tested by a restricted likelihood t-test using Satterthwaite approximations for degree of freedom.

RESULTS

Behavioural repeatability

The strength of repeatability depended on behavioural trait and age (Fig. 4). In risk-boldness and foraging-boldness, geckos showed high repeatability at the age of 0.2 and 6 months (ICC ranges 0.60 to 0.75) but low repeatability at the age of 3, 9, 12, and 15 months (ICC ranges 0.20 to 0.35). In activity, geckos in all age classes showed high repeatability (ICC ranges 0.50 to 0.80). Overall, the strength of consistent behavioural differences in risk-boldness and foraging-boldness were diminished at a specific period of juvenile stage and most periods of adult stages. On the other hand, activity was a stable personality trait for clonal geckos throughout their lifetimes.

Behavioural syndromes

Geckos showed correlations between behavioural traits at juvenile stages (0.2–6 months) but not at adult stages (9–15 months) (Table 3). However, combination of behavioural traits and its direction (positive or negative correlation) changed with age. Geckos at the age of 0.2 month showed a negative correlation between risk-boldness and foraging-boldness (r_S = -0.48, p = 0.014), but geckos at the age of 6 months showed a positive correlation between these behavioural traits (r_S = 0.47, p = 0.019). In other combinations, a moderate correlation between foraging-boldness and activity was detected in only geckos at the age of 3 months (r_S = 0.45, p = 0.023).

390

391 *Behavioural stability across age classes*

392 In most cases, a stable behavioural tendency over the ontogeny was not found
393 between two adjacent age classes (Table 4), representing that bolder
394 individuals at early stage were not always bolder at later stage. The exceptions
395 were risk-boldness across the age of 3–6 and 12–15 months, and foraging-
396 boldness across the age of 12–15 months where a moderate positive
397 correlations were detected (r_S ranges 0.47 to 0.65).

398

399 *Effect of food control*

400 For life-history traits, the different amounts of food caused differences of pace-
401 of-life between the two groups. Until the age of 9 months, the high energy group
402 showed high growth rates than the low energy group (Table 5). After the age of
403 12 months, the high energy group stopped growing but the low energy group
404 showed a compensatory growth (Fig. 5a, b), reflecting the significant differences
405 of growth rate across the age of 12–15 months (Table 5). In reproduction, the
406 high energy group laid eggs at younger age than the low energy group (t =5.05,
407 df =18.4, p < 0.001) (Fig. 5c).

408 For behavioural traits, no significant differences between the high and low
409 energy groups were detected in foraging-boldness and activity at the age of 3–
410 15 months (Fig. 6). In risk-boldness, significant difference between the two
411 groups was detected at the age of 3 months; the low energy group tended to be

412 shyer than the high energy group. However, this difference was diminished at
413 older age classes.
414
415 DISCUSSION
416
417 The longitudinal observations of clonal geckos revealed three characteristics of
418 their behavioural traits over the ontogeny. First, the strength of repeatability
419 varied with age and among behavioural traits. Second, the structure of
420 behavioural syndrome changed at several life stages. Third, the rank order of
421 behavioural tendencies among individuals were not always maintained
422 throughout their development. These findings suggest that, in clonal geckos, the
423 presence and structure of personality are unstable from hatching to sexual
424 maturation under the laboratory condition. Recently, several researchers have
425 reported similar findings: age dependent behavioural repeatability (Petelle et al.
426 2013; Urszán et al. 2015; Polverino et al. 2016), life stage specific behavioural
427 syndromes (Class and Brommer 2015; Favati et al. 2016), and unstable
428 behavioural tendencies over the life time (Sinn et al. 2008; Favati et al. 2016;
429 Riley et al. 2017). The mechanisms and adaptive significance of the labile
430 personality throughout the ontogeny still remain unclear (Stamps and Groothuis
431 2010). In the following sections, based on the present results, I discuss
432 applicability of the previous models on personality formation and a possible
433 mechanism that generates this “life stage specific” behavioural variation.
434

Negative support for the previous models

The different amounts of food supply induced the significant differences of pace-of-life among clonal geckos in growth rate and reproduction. However, significant behavioural differences between the low and high energy groups were not detected in most ages. These results suggest that neither the POLS nor the asset protection models are valid to the personality formation of clonal geckos. Why state variables via energy intake did not bring about strong effects on gecko's behaviours? The weak associations between state and behaviour may be attributed to three non-exclusive possibilities: (1) methodological limitations, (2) physiological characteristics of ectotherms, and (3) theoretical validation.

First, in the present study, differences in intrinsic energy state may be too small to influence behavioural traits. State-behaviour feedbacks assume that slight differences of state variable trigger behavioural change, but the strict definition of "slight differences" was not mentioned in the previous frameworks (Sih et al. 2015; Stamps and Biro 2016). In addition, positive feedbacks predict that differences of energy intake gradually increase with time (e.g., 1.1 times at early stage but over 3.0 times at later), but I maintained differences of food supply consistently as 1.5–1.6 times between the two groups. For further studies, it should be considered that how much degree of state differences between individuals is sufficient to influence behavioural traits.

Second, physiological characteristics of ectotherms may further weaken the differences in energy state between the two groups. Reptiles show a relatively

low basal metabolic rate compared to mammals and birds (Pough 1980), and they allocate a large proportion of redundant energy to growth and reproduction (Congdon et al. 1982). Thus, in reptiles, differences of the absolute energy expenditure for the basal metabolism seem to be small between individuals of different body size (Nagy 2005). Because the basal metabolic rate is the key driver of the POLS, its large differences are essential for inducing behavioural variation (Biro and Stamps 2010; Mathot and Dall 2013). In the present study, different amounts of food may have induced different levels of redundant energy, which reflected significant differences of life-history traits between the two groups. However, the basal metabolic rate of the both groups may have been maintained at low levels irrespective of different energy intake, which may have caused small differences in behavioural traits.

The third explanation is that the POLS and asset protection models might be not the general principle for many organisms to explain their intraspecific behavioural variation. Recent meta-analyses revealed a relatively low effect size of support for the POLS model (Royauté et al. 2018) and weak association between intrinsic states and behavioural traits (Niemelä and Dingemanse 2018). The present study provides an additional case demonstrating that, even among clonal individuals, behavioural variation is not simply explained by individual's energy state. Further verifications and reconsiderations of these models should be required (Mathot and Frankenhuis 2018).

Life stage specific effect of state on behaviour

481 The only significant effect of food control was detected in risk-boldness, and the
482 observed pattern seems to be matched with another concept: state-dependent
483 safety model. This model assumes that escape ability from predators depends
484 on individuals' state and that individuals change risk-taking propensity based on
485 their escape ability (Luttbeg and Sih 2010). Several studies have reported that
486 individuals with good state cope better with predators by fleeing faster and
487 defencing better (Temple 1987; Lindström et al. 2006; Alzaga et al. 2008;
488 Hoefler et al. 2008). Individuals with good state should be bold because
489 foraging effort itself enhances state and further safety, whereas individuals with
490 bad state should be shy because predation risk overwhelms benefit of the
491 increase of state in foraging situation (Luttbeg and Sih 2010). Therefore, in the
492 present study, the high energy group may show risk-prone tendency because of
493 their better state and escape ability than the low energy group. However, the
494 developmental trajectories suggest that the state-dependent safety model does
495 not simply lead to divergence of risk-boldness throughout the gecko's life.
496 Considering that ecological features change with the development, "life
497 stage specific" state-dependent safety would be reasonable to explain the
498 present results. Because small juvenile geckos neither run fast nor defend
499 themselves from predators, they are vulnerable to small carnivorous
500 invertebrates such as spiders and centipedes (Bauer 1990). In addition, geckos
501 cannot consume prey animals larger than their gape size, meaning that
502 available food resource of small juveniles is restricted compared with larger
503 adults (Urban 2007). With increasing body size, *L. lugubris* is released from

some predators and can consume larger prey animals, which decreases predation risk while increases resource availability. A recent theoretical model demonstrated that the magnitude of state-dependent safety is balanced by predation risk and resource availability; middle levels of risk and resource availability lead to a divergence of boldness based on individual's state, but low risk and high resource availability lead to a convergence of boldness regardless of individual's state (Luttbeg and Sih 2010). When the natural history of *L. lugubris* (i.e., predation risk reduces but food availability increases with the development) is applied to this model, it predicts a divergence of boldness in the stage of small juveniles but a convergence of boldness (all geckos behave bold) at their later life stages.

Conclusions

The personality formation of clonal geckos does not support the POLS or the asset protection models. In these geckos, the effect of energy state on behavioural traits was life stage specific, suggesting that dominant mechanisms of personality formation may change throughout their lifetimes. Overall, even when clonal animals are reared under the controlled environment, personality formation seems to be a more complicated process than the predictions of previous simple models (Réale et al. 2010; Sih et al. 2015).

On the other hand, in the previous study of free-living *L. lugubris*, a larger behavioural variation was detected in adults than in juveniles (Sakai 2018). However, in the present study, behavioural variation increased at the juvenile

stage but decreased at the adult stage. This deviation between wild and laboratory populations may reflect the effects of other environmental factors such as threat experiences (Bell and Sih 2007), social interactions (Riley et al. 2017), and thermal conditions (Siviter et al. 2016; Michelangeli et al. 2018). For further studies, longitudinal observations of behavioural traits under semi-natural environment are desired to understand how personality variation increases, or sometime decreases, in more complicated environments.

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ETHICAL NOTE

The present study was carried out in compliance with the guidelines of the Animal Care and Use Committee of Kyoto University. Experiment and husbandry conditions were designed to minimize stress and harm in subjects.

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734

735 TABLES

736 **Table. 1** Feeding protocol for the low and high energy groups throughout the
737 development. Both groups consistently consumed different amounts of food
738 (crickets) until subjects reached the age of 12 months, except before the first
739 behavioural test at the age of 0.2 month, during when I provided the same
740 amount of food (50 mg) to all subjects. After the age of 12 months, both groups
741 consumed the same amount of food until the first reproduction.

742

Age (month)	Food supply (mg/week)		Ratio (High/Low)
	Low energy group	High energy group	
0.2–3	50	80	1.60
3–6	80	120	1.50
6–9	140	210	1.50
9–12	210	320	1.52
12 <	320	320	1.00

743

744

745 **Table. 2** Results of principal component analysis for behavioural traits in each

746 assay. I used the data set of 441 observations for 25 individuals in both assays.

Assay type	Behavioural variable	PC1	PC2
Threat assay	Total hiding duration (min)	-0.71	0.11
	Latency to appearance (min)	-0.70	-0.21
	The number of refuge use	-0.07	0.97
	Propotion of Variance	0.62	0.35
Food assay	Total duration at food area (min)	0.51	-0.05
	Latency to approach (min)	-0.61	0.29
	The number of entering to food area	0.52	-0.16
	The number of strikes to the cup	0.30	0.94
	Propotion of Variance	0.51	0.23

747

748

749 **Table. 3** Behavioural correlation between risk-boldness, foraging-boldness, and
750 activity at six age classes. Bold letters indicate significant correlation at $p <$
751 0.05.

Age (month)	Combination of behavioural trait					
	Risk-boldness × Foraging-boldness		Foraging-boldness × Activity		Risk-boldness × Activity	
	r_s	p	r_s	p	r_s	p
0.2	**-0.48**	**0.014**	0.14	0.536	-0.03	0.907
3	0.21	0.321	0.32	0.113	**0.45**	**0.023**
6	**0.47**	**0.019**	0.11	0.601	-0.34	0.094
9	0.37	0.074	0.04	0.859	0.02	0.929
12	0.22	0.309	0.04	0.862	0.23	0.272
15	0.12	0.572	-0.10	0.639	0.23	0.287

752

753

754 **Table. 4** Behavioural stability between two adjacent age classes for three
755 behavioural traits. Positive significant correlation represents that rank order of
756 individuals is maintained over three months. Bold letters indicate significant
757 correlation at $p < 0.05$.

Age (month)	Behavioural trait					
	Risk-boldness		Foraging-boldness		Activity	
	r_s	p	r_s	p	r_s	p
0.2–3	0.11	0.617	**-0.45**	**0.025**	0.16	0.484
3–6	**0.47**	**0.018**	-0.17	0.407	0.31	0.134
6–9	0.26	0.225	0.28	0.179	0.19	0.378
9–12	0.15	0.474	0.34	0.109	0.20	0.356
12–15	**0.65**	**0.001**	**0.50**	**0.015**	0.34	0.104

758

759

760 **Table. 5** Growth rate in body mass (mean ± SD) of the low and high energy
761 groups during five time periods across the ontogeny. Bold letters indicate
762 significant differences at $p < 0.05$.

Age (month)	Feeding group		*t*	*df*	*p*
	Low energy	High energy			
0.2–3	0.06 ± 0.11	0.21 ± 0.11	-3.31	19.1	**0.004**
3–6	0.27 ± 0.05	0.31 ± 0.04	-2.06	23.0	**0.050**
6–9	0.06 ± 0.11	0.21 ± 0.11	-3.31	19.1	**0.004**
9–12	0.16 ± 0.03	0.17 ± 0.04	-0.70	21.3	0.489
12–15	0.15 ± 0.02	0.00 ± 0.06	8.79	16.4	**<0.001**

763

764

765 FIGURE LEGENDS

766 **Figure. 1** Conceptual frameworks of the state-behaviour feedback hypothesis
767 based on the (a) pace-of-life syndrome (POLS) and (c) asset protection models.
768 Black allows represent possible effects of energy intake on state variables, life-
769 history traits, and behavioural traits. Although the feedback loop is achieved by
770 effects of behavioural traits on energy intake (grey allows), to simplify the
771 mechanisms, the present experiment is designed to investigate only the effects
772 represented by black allows. In the effect of the different amounts of food on
773 behavioural traits, opposite behavioural changes throughout the development
774 are predicted by the (b) POLS and (d) asset protection models. Green lines
775 represent trajectory of the high energy intake group and blue dashed lines
776 represent trajectory of the low energy intake group.

777

778 **Figure. 2** Experimental schedule throughout the life stage of *L. lugubris*. Black
779 triangles represent the timing when behavioural tests were conducted every
780 three months of subject's age. At each point, I conducted a series of threat,
781 food, and activity assays during eight days.

782

783 **Figure. 3** Experimental arenas for three types of assays. In the threat assay (a),
784 grey colour represents that the assay was conducted in a dark room. In the food
785 assay (b), a shaded block represents a food area in which the cup containing 10
786 live crickets was located. In the activity assay (c), dashed lines represent infra-
787 red laser beams of the sensor system.

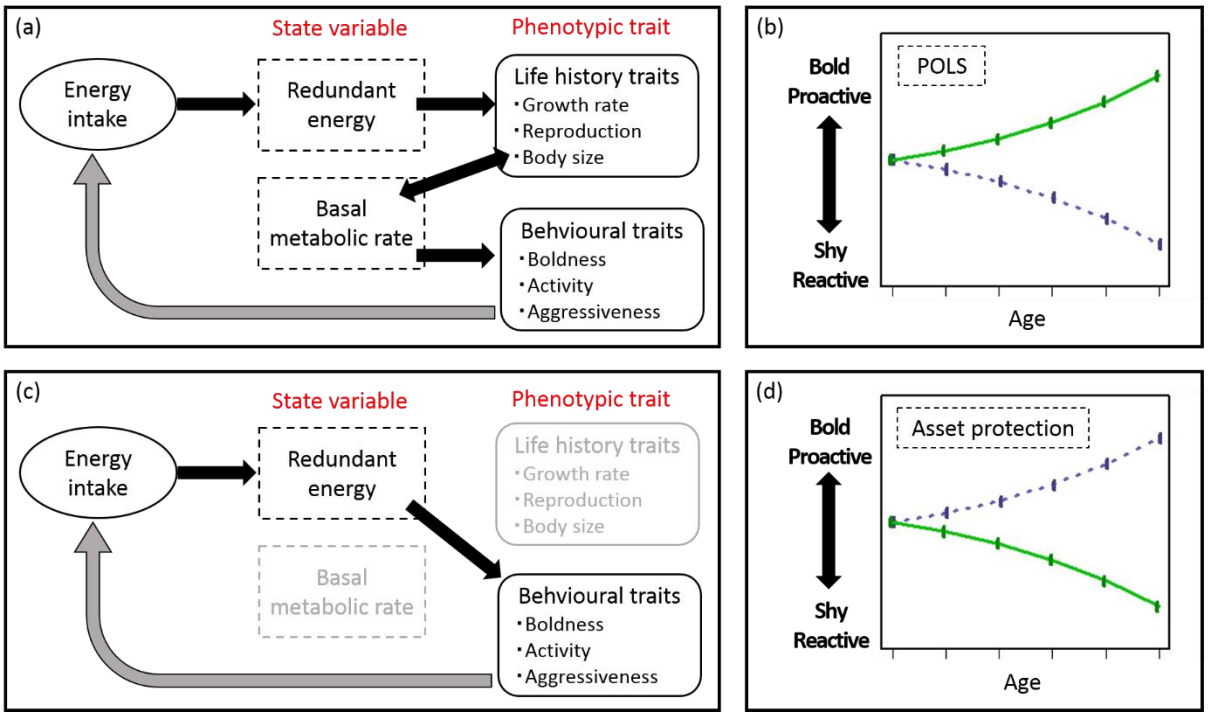
Figure. 4 Estimate with 95% confidence intervals of behavioural repeatability in three behavioural traits at six age classes (N = 25 individuals for the age of 0.2–6 months; N = 24 individuals for the age of 9–15 months). Based on log likelihood ratio tests for the random effect of individual identity, significance of repeatability is indicated by *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Figure. 5 Comparison of pace-of-life between the high (green, N = 14) and low (blue, N = 11) energy groups. In (a) snout-vent length (SVL) and (b) body mass, mean and SD are represented by the centre and length of bars, respectively. In (c) age at the first reproduction, box plots represent median, 1st and 3rd quantiles, and outlier value.

Figure. 6 Comparison of behavioural tendencies between the high (green, N = 14) and low (blue, N = 11) energy groups throughout the life stage. Based on t-test using Satterthwaite approximation for degree of freedom, the significant effect of energy intake is indicated by *: $p < 0.05$, **: $p < 0.01$.

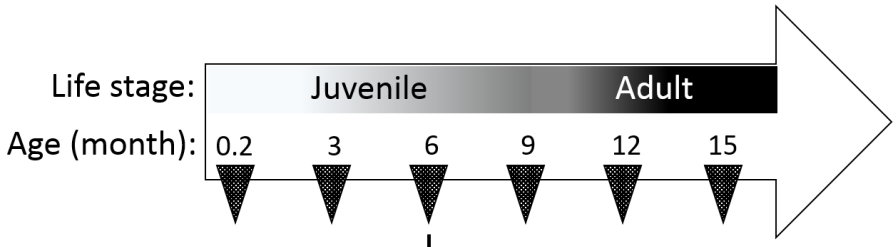
806 FIGURES

807 Figure.1



808

809 Figure.2

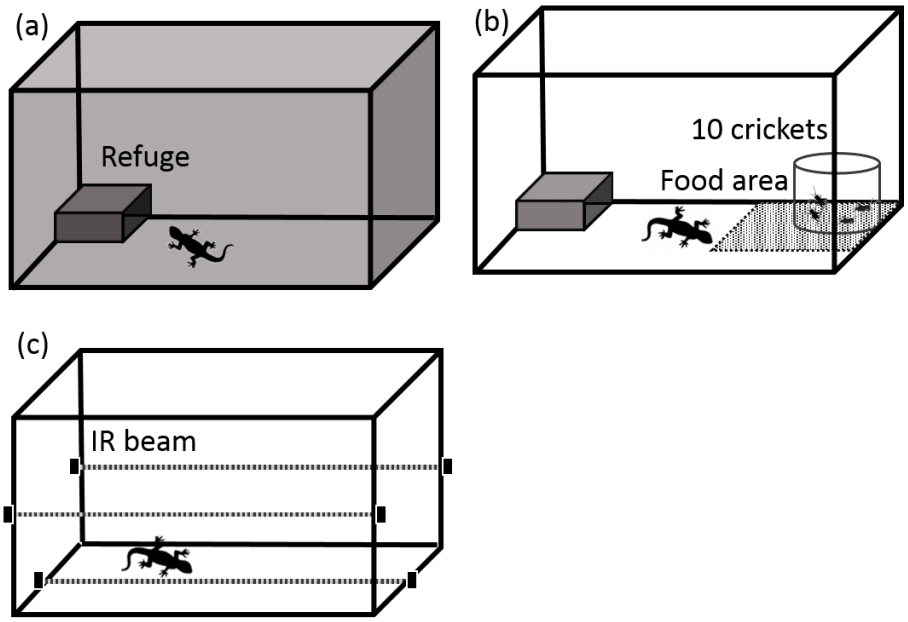


Schedule at test week

Day	Manipulation
1	Feeding
2-4	Threat assay & Food assay
5-7	Activity assay
8	Measurement & Feeding

810

811 Figure.3



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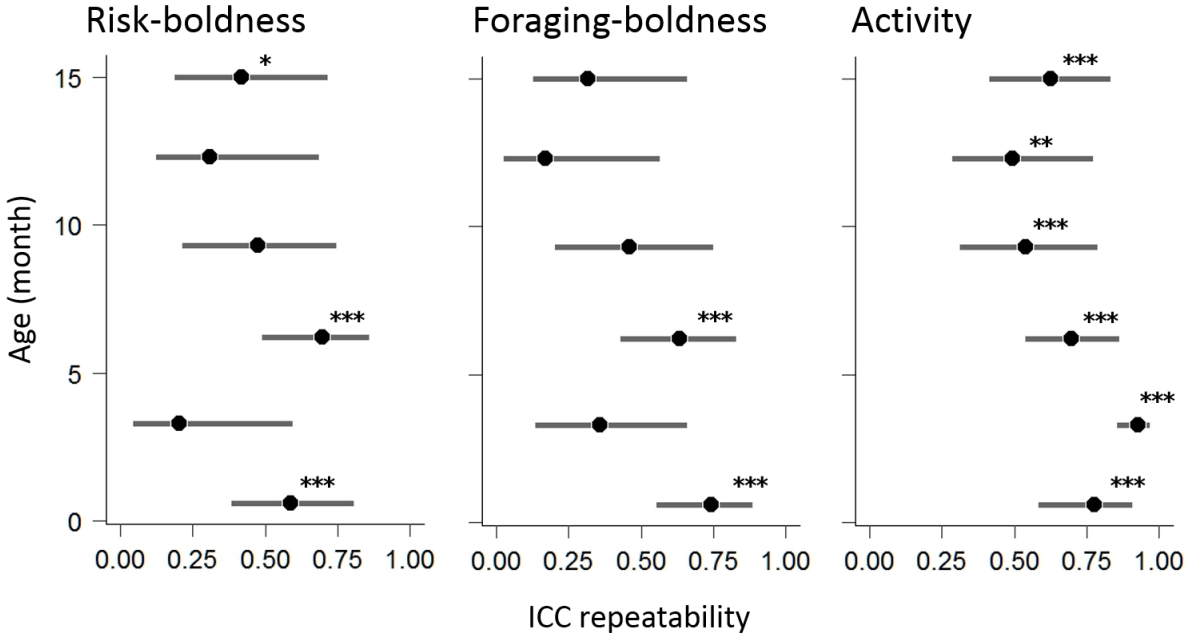
813

814 Figure.4



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816 Figure.5

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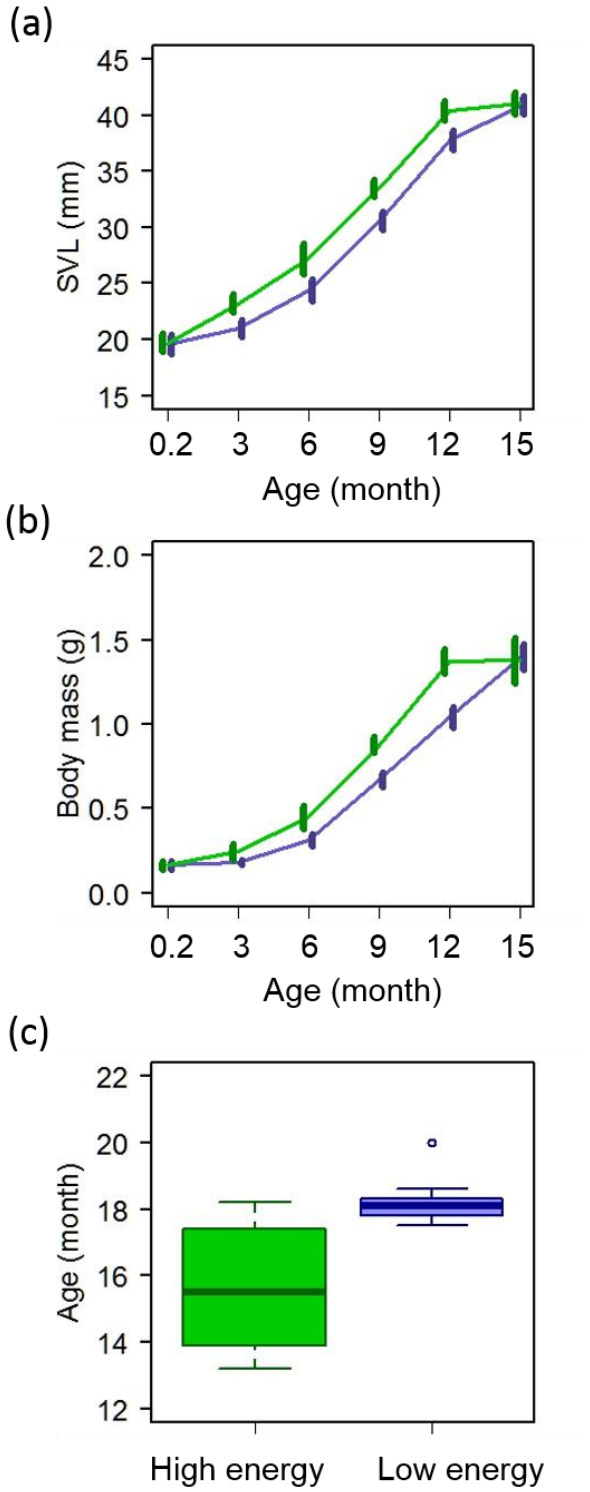

819 Figure.6

820