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Title

Sympatric divergence of risk sensitivity and diet menus in three species of tit

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Abstract

Divergent diet menus could cause sympatric divergence of risk sensitivity, however, evidence is not yet available for the functional link between them. In this study, risk sensitivity (measured as the discount intensity for probabilistic rewards) and diet menu (insectivory and granivory) among three sympatric species of tits (family Paridae) was investigated. These species—varied tits (*Poecile varius*), marsh tits (*Poecile palustris*) and great tits (*Parus major*)—form mixed-species foraging flocks in Japan. Binary choice tests, offering rewards of differing amount and probability, were conducted in the laboratory. Great tits and marsh tits were found to be risk prone (and more insectivorous), whereas varied tits were risk averse (and more granivorous). Diet menus were examined in the laboratory using behavioural titration tests between sunflower seeds and mealworm. The results of these tests were similar to patterns of food exploitation determined using stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ contents) of blood samples from birds collected in the wild. It is possible to assume that the dominant varied tits drove the other two species of tits towards different diet menus and unusually high risk proneness. In future, we should examine if different inter-species interactions causes different risk sensitivity in other geographical environments.

Keywords:

decision making, food ecology, mixed species flock, resource competition, social foraging, Bayesian estimation

Food resources vary and are inevitably probabilistic in nature. Foraging animals thus face a risk of not gaining the food items that are anticipated. In a wide range of taxa (from bees, fish, birds to mammals), animals generally show risk aversion, but may show risk proneness when the energy budget is negative (Caraco et al. 1980; Kacelnik & Bateson 1996). Theoretically, it has been shown that such energy-status dependent risk sensitivity is adaptive, because it decreases the probability of starvation (Stephens 1981; McNamara & Houston 1992).

However, divergent risk sensitivity could occur independently of energy status. Recently, Heilbronner et al. (2008) compared chimpanzees and bonobos in captivity and found that they differ in risk sensitivity. Chimpanzees are risk prone, whereas bonobos are risk averse, even in identical experimental conditions with the same energy budgets. Heilbronner and colleagues explained the divergence in terms of different feeding ecology, by citing Wrangham & Peterson (1996) that bonobos rely more heavily on terrestrial herbaceous vegetation (THV) than hunting and fruit-eating chimpanzees (also see Gilby & Wrangham 2007). Such divergence of diet menus could be caused by inter-species competition with gorillas, which depend largely on THV for their diet (Malenky & Wrangham 1994). The absence of gorillas on the south bank of the Congo River means that bonobos do not face this competition and can depend more on THV relative to chimpanzees. Conversely, chimpanzees reside on the northern river-bank and share the habitat with gorillas. It could be argued that chimpanzee risk proneness occurs because of diet menu competition with gorillas, yet no direct evidence currently exists.

To elucidate whether divergent diet menus cause sympatric divergent risk

sensitivity, it is critical to ask if divergent risk sensitivity is correlated with the respective diet menus among sympatric species. As the first step to answer this question, the present study focused on three sympatric species of tits; varied tits (*Poecile varius*), marsh tits (*Poecile palustris*), and great tits (*Purus major*). Taxonomic studies of mitochondrial DNA have revealed that marsh tits are closer to varied tits than to great tits (Gill et al. 2005). These three species of tits coexist in relatively isolated areas of eastern Asia, such as Hokkaido in Japan and the Korean peninsula (Jabłoński & Lee 1998, 2002; Park et al. 2005). These three tit species form mixed-species foraging flocks (together with *Periparus ater* and *Aegithalos caudatus*) (Nakamura 1967; Jabłoński & Lee 1998, 2002), particularly during the harsh winter season when food is scarce. According to the general rule that the dominance hierarchy is correlated with body size in mixed-species flocks (Gibb 1954; Morse 1970, 1978; Hogstad 1978; Jabłoński & Lee 2002), varied tits are thought to be dominant over great tits in Japan (Urata & Ueda 2003). They also differ in their food storing behaviour; varied and marsh tits store food (varied tits, Higuchi 1975; marsh tit, Stevens & Krebs 1986), while great tits do not (Sherry 1989). All three species of tits are omnivorous, but field observations have reported that varied tits are more granivorous whereas great tits tend to be more insectivorous (Nakamura 1970).

METHODS

Subjects

A total of 26 tits (9 varied tits: *Poecile varius*, 8 marsh tits: *P. palustris* and 9 great

tits: *Parus. major*) were used (see Appendix Table A1). All birds were captured using mist nets or box traps at Sapporo, Japan (experimental field station of the Hokkaido University, Sapporo Campus; N43°4'18", E141°20'29") during winter (December to March) in 2008–2011 under the approved licences issued from Ministry of the Environment of the Japanese Government (licence # 02-0341) and from the Natural Environmental Division of the Hokkaido Government (licence # 187). Coloured plastic leg rings were used to identify individuals. Blood (ca. 50µl) was sampled through the wing vein using 25-G syringe needles, and used to verify the sex of individuals based on the CHD genes of the sex chromosomes (Fridolfsson & Ellegren 1999). Blood samples were also used for stable isotope analysis (see below for details). Of the 26 birds, 8 were re-released into the field immediately after blood sample collection. The rest (18 birds) were kept in captivity for behavioural experiments in the laboratory under the guidelines and licence of the Hokkaido Government and the Hokkaido University. The guidelines are based on the national regulations for animal welfare in Japan (Law of Humane Treatment and Management of Animals; after a partial amendment No.68 of 2005).

The captured tits were individually housed in metal cages (42 × 35 × 66 cm) and fed with a mixture of commercial food (mixed grains and fish meat), fresh vegetables and egg yolk supplemented with multiple vitamin compounds. Food trays were replenished every day with fresh food, except for the egg yolk, which was supplied once a week. In addition, fresh mealworms, sunflower seeds and hemp seeds were given once every 2 days. Water was always available ad libitum. Food was freely available except

for the 2–3 hours prior to testing. We assumed that the daily energy budget was surplus to requirements even when tits were temporally deprived of food prior to testing. To avoid stress from handling, body weight was measured twice (at the time of capture and at the end of the experiments). These two measurements of body weight were averaged for individual tits. The food tray was weighed every day to check how much food was consumed on the previous day. In this study, no birds showed indications of starvation or stopped foraging in captivity.

The cages were placed in a quiet laboratory room. The temperature ranged between 16–27 °C. Housed in individual cages, the birds were physically isolated but not socially, as they could visually/acoustically communicate. Artificial lighting was not used and the photoperiod was not artificially controlled but rather followed the natural sunset-sunrise cycle of light which came through the wide glass windows of the laboratory. After the study, the birds were housed in a communal outdoor aviary (4m × 8m, 3m high; at the experimental field station and original capture site) and fed the same diet until release the following spring. At the time of release the birds were in good condition and actively foraging.

Apparatus

All behavioural tests used binary choice devices (12 × 8 × 3 cm) placed in the subject's home cage (Fig. 1a). In each device, two identical food trays were visible (through a transparent acrylic plate) but not accessible. A string attached to each of these food trays allowed the tit to select a food tray by pulling the string. A third string

connected to both food trays ensured only one tray could be selected. When the string for one tray was pulled, the other tray was automatically withdrawn and the stopper bar inside dropped. Upon selection of a food tray, the subject tit then pulled the coloured cover off the tray to retrieve the food.

Figure 1 around here

The colours and patterns on the cover of each tray indicated the properties of the food reward, i.e., the amount and the probability. To examine risk sensitivity (Fig. 1b), freshly cut pieces of mealworm were used as the reward (20–40 mg per piece). For example, a tray with a pink/green cover (labelled as 3A(1)) yielded 3 pieces of mealworm at probability = 1, thus it was a no-risk option. On the other hand, trays with red/blue and orange/brown covers yielded the same amount of food but at probability (P) = 2/3 (high-probability) and 1/3 (low-probability), respectively. Yellow and white covers cued a no-risky option of one piece of mealworm (A(1)) and no-reward (S-), respectively. To examine food preference (Fig. 1c), trays with different food rewards were used: trays with orange/purple cross-patterned covers contained one sunflower seed (with husk; 95–180 mg); trays with yellow/green covers contained mealworm pieces where the number of the pieces (A) varied among trials (see below for details), whilst trays with white covers always indicated that the tray contained no food (S-). After training, tits were tested with pairs of reward options. In this study, colour assignments were fixed, and a particular colour was always used to cue a particular reward in all subjects. If colour preference existed, and it differed among tit species, our experimental design might give rise to biased evaluations of the reward values.

Experiment 1: Risk sensitivity

Training procedure

Prior to tests, tits received training blocks consecutively for 3–5 weeks, and the number of blocks did not differ among tit species; 18.5 ± 3.4 blocks in varied tits (mean \pm s.e.m., $n = 6$), 13.7 ± 0.7 blocks in marsh tits ($n = 6$), and 14.5 ± 1.3 blocks in great tits ($n = 6$). One block was performed per day, each composed of a total of 40 trials; i.e., A(1) vs. S- in 15 trials, option X (= 3A(1), 3A(2/3) or 3A(1/3)) vs. S- in 15 trials, and A(1) vs. option X in 10 trials. In the initial phase of training, 3A(1) was always used for option X. Once the subject tit reached certain training criteria (see below), 3A(2/3) and 3A(1/3) were subsequently used for option X. The training blocks continued until each subject reached the criteria in all of the rewarding options.

The side (reward placement in the right or left food tray) was pseudo-randomly balanced within each block. Tits reached the required response ratio (i.e., the ratio of trials in which a reward option (A(1) or option X) was chosen against no reward (S-)) of greater than 0.8 within 2–5 days. However, in trials with A(1) vs. option X, tits generally showed a side bias. Some tits persistently chose the left (or the right) food tray, even though the side of presentations was counter-balanced. Training blocks were therefore continued until the side bias disappeared and the ratio of the trials in which the right food tray was chosen fell in a range of 0.25–0.75.

Test procedure

Subject tits were tested for all possible binary choice combinations (${}^4C_2 = 6$ combinations) during the spring–summer of 2010/2011 (see Table S1 for subject list), when the choice options for 18 tits (six individuals for each species) were recorded. The S- option (tray with a white cover that cued empty reward) was included only in training trials in the test block (see below) in order to confirm that tits consistently chose rewarding options. For each combination and each subject, tests were replicated four times. Thus a total of 24 test blocks (6 combinations each replicated 4 times) were arranged in a pseudo-random order. Each subject was given one test block per day and it took 1–2 months to complete the 24 test blocks. Since mealworms could be a nutritionally biased meal, subjects were tested on six or less days per week. The total amount of mealworm gained in a test block constituted ca. 20–30% of the total diet that tits consumed each day. One block was composed of a consecutive series of short training trials (six binary choices between a reward option vs. S-) followed by another consecutive series of trials (12 test trials between two rewarding options, pseudo-randomly arranged with 18 training trials between one of the rewarding option paired with S-). The side (right or left food tray) was pseudo-randomly balanced within each block. The number of each choice type was averaged over the four replicate blocks (i.e., 48 trials in total) to provide substantial data for individuals.

Statistical analysis

To analyse behavioural data, we constructed hierarchical Bayesian models on the basis of the cognitive mechanisms that were assumed to underlie the observed choice

behaviour. This method enables us to estimate hidden cognitive parameters that we cannot directly observe (such as subjective value and its dependence on food probability) from behavioural data (such as probability and amount of food and choices made by animals) through constructing statistical models that include nested structures and two or more random effects. The following two characteristics of the hierarchical Bayesian model are important. (1) Each parameter is estimated in form of a most likely distribution rather than as a single scalar value. And (2) the models are hierarchically constructed, so that estimated distribution (referred to as *posterior distribution*) follows a *prior distribution*, which further follows a *hyper-prior distribution* that is *a priori* given as non-informative, flat distribution. It should be noticed that the *prior* distributions may implicitly include some plausible assumptions, e.g., unbiased colour preference at population level; see below for the issue of colour biases.

A Markov chain Monte Carlo method (MCMC) was applied to a hierarchical Bayesian model to estimate the subjective value and other cognitive parameters (See McCarthy 2007 for construction of Bayesian models). The mathematical models are briefly described below. For further details, see the previous report (Kawamori & Matsushima 2010). After the prior and posterior distributions of the inferred parameters were calculated, we examined the plausibility of the model assumptions by simulating the data for choice from the fitted posterior predictive distributions. We then cross-checked if the simulated data fitted the observed behavioural data.

The choice ratio (calculated on the basis of observed behavioural data) was used as the response variable. Since birds were tested with binary choices, it was assumed

that the error structure of the choice ratio followed a binomial distribution with a choice probability = $Q(X)$ ($\in [0, 1]$). This was approximated by a logistic function:

$$Q(X) = 1/(1 + \exp(-X)) \quad (1)$$

in which a linear predictor X was set as a weighted difference of values (ΔV) for each individual (i) with a combination of choice options (c). Here, ΔV denotes the difference of subjective values (inferred parameter; see below for details) between two options of the binary choice in test.

$$X_{i,c} = \text{Bias}_{i,c} + \text{Coefficient}_i \times \Delta V \quad (2)$$

$\text{Bias}_{i,c}$ denotes the innate bias consistently produced by each individual for each combination, thus is an inferred parameter. $\text{Bias}_{i,c}$ was assumed to follow a non-informative prior distribution *e.g.* a Gaussian distribution, which was defined with 0 for the mean and 10 for the variance.

Coefficient_i represents how strongly the difference of values affects choice, thus is also an inferred parameter. It was determined using a Gaussian prior distribution. The hyper-prior distributions (the prior of prior distribution) were given by non-informative distributions.

The subjective value (V) was defined for each individual/species with each option as:

$$V_{i,s} = \text{Amount} - \text{Discount}_{i,s} \quad (3)$$

where *Amount* is 1 or 3 (pieces of mealworm) and is an experimentally controlled parameter. The posterior probability density of $\text{Discount}_{i,s}$ was computed for each individual and species within this framework. Here, we assumed species-specific prior

distributions that produced samples for individual posterior distributions. It is a fundamental question whether such a value could be uniquely given, but the behavioural data from this study were not contradictory to this idea, because the transitivity of choices was not violated (see Results)

Discount_{*i,s*} was further defined as a product of two independent variables, both of which are inferred parameters in this model:

$$\text{Discount}_{i,s} = \text{Intensity}_{i,s} \times \text{Skewness}_s \quad (4)$$

Intensity_{*i,s*} represents how strongly individuals devalued food when faced with the low probability ($P = 1/3$), thus gives rise to a measure of risk aversion in units of mealworm pieces. Species specific prior distributions produced samples for individual posterior distributions. The hyper-prior distributions were given by non-informative distributions.

Skewness_{*s*} is a variable that characterizes the shape of the Discount_{*i,s*} function particularly for the higher probability ($P = 2/3$). It was assumed that Discount_{*i,s*} was a non-linear monotonically non-increasing function of the probability of food (P). In other words, we assumed that a higher P caused a smaller Discount_{*i,s*}, with Discount_{*i,s*} = 0 at $P = 1$. Skewness_{*s*} was thus assigned to 0, α , 1 for $P = 1, 2/3, 1/3$, respectively, and the value of α ($\in [0, 1]$) indicated how strongly the option value was discounted for $P = 2/3$ in each species. The model was too complex to estimate α for each individual from the current data, and thus it was assumed that α had no inter-individual variance within a species. The prior distribution of α was given as a uniform distribution in a range 0 to 1.

Experiment 2: Food preference

For food preference the degree of granivory was investigated. Binary choice tests between mealworms and one sunflower seed were used. The relationship between food preference and risk sensitivity among individuals of the three species was analysed. A behavioural titration procedure was employed, such that the number of worm pieces was adjusted toward an equilibrium point at which a seed and worm pieces were equally chosen. One great tit died before the second experiment and thus data were obtained from a total of 17 tits (six varied and marsh tits and five great tits).

Experimental procedure

The association between the colour patterns and food rewards is shown in Fig. 1c. Birds were given binary choices between one seed and A pieces of worm. In the first block $A = 3$ was assigned. In the 2nd and subsequent blocks, the number of worm pieces (A) was adjusted in each block depending on the individual's choice in the preceding block. Each block was composed of training trials (four choice trials paired with S- (white); two for each reward option, arranged in pseudo-random order) followed by test trials (three binary-choice trials). The side placement of food options (right or left food tray) was balanced in each block for training trials, and in each two blocks for test trials. If a subject chose the seed more frequently than the worm (i.e., the choice of worm = 0 or 1) in block t , then the number of worm pieces was incremented by +2 or +1, respectively, in block $t+1$. On the other hand, if the subject chose the seed less than the

worm (choice of worm was 2 or 3) in the block t , then the number of worm was decreased by -1 or -2, respectively, in block $t+1$.

Statistical analysis

Another hierarchical Bayesian model was developed to examine the correlation between risk sensitivity and food preference. Since a single test block had only 3 choice tests, the observed numbers of choices (ranging in 0–3) fluctuated too much to directly represent the subjective value. Thus, increase/decrease of the number of mealworms in the next block was used as the response variable, rather than the number of mealworm pieces (A) per se. The response value was given as either 0 (decrease) or 1 (increase) following a Bernoulli distribution with a choice probability = $Q(X)$ ($\in [0, 1]$) which was approximated as

$$Q(X) = 1/(1 + \exp(-X))$$

in which a linear predictor X was given for each individual (i) and block (b) as the difference between the subjective value of one sunflower seed (SF_i) and the objective number of mealworm pieces at each block (MW_b):

$$X_{i,b} = SF_i - MW_b \quad (5)$$

Here, SF_i was assumed to correlate with $Intensity_i$, where $Intensity_i$ represented the risk sensitivity measured for the low probability option, $P = 1/3$. SF_i was calculated as:

$$SF_i = \text{Gradient} \times Intensity_i + \text{Intercept}_i \quad (6)$$

Since Intercept_i could move freely, allowing SF_i to fit individual preference data, the estimated Gradient was assumed to precisely represent the regression coefficient.

Both Gradient and Intercept_{*i*} were assumed to follow non-informative prior distributions.

Stable isotope analysis for food exploitation

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope signatures of animal tissues provide reliable information about the food consumed by an animal under study (DeNiro & Epstein 1978, 1981; Minagawa & Wada 1984; Mizukami et al. 2005; Rutz et al. 2010). In general, insectivorous animals have a lower $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratio than granivorous animals. Thus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from blood samples were compared among the three species of tits and compared with the possible food items available in the capture sites using stable isotope analysis.

Blood samples of wild tits

Blood was sampled from 18 tits (five varied tits, four marsh tits, nine great tits; see Table S1) immediately after birds were captured. It should be noted that birds were captured in winter (between December and March), i.e., when mixed foraging flocks and competition for resources between tit species occurs. As a reference, blood was also sampled from the tits kept in the laboratory and fed with laboratory food for 16–30 months; three great tits, four marsh tits and four varied tits. As shown in Fig. 5, few differences were found among these laboratory-raised tits. The observed differences in the wild tits can thus be ascribed to different feeding patterns, rather than metabolic differences of the digestive system.

Food samples

Possible food items (invertebrate animals and plants) were collected from the Sapporo campus of the Hokkaido University, where the tits were captured, and classified into the following three categories (see Appendix Table A2). (i) Small invertebrates (11 species of insects consisting of 10 families in four orders, three species of spider consisting of three families in the same order, one species of earthworm, and one species of snail). These wintering invertebrates were collected from tree trunks and the ground surface. (ii) C3 plants (five species of seeds from five families in five orders, two species of fruits from two families in the same order). These items were collected in an area within 1 km of the birds' capture site. (iii) Seeds from commercial, C4 plants (three species in the Poaceae family) were also included. The C4 plants were separated from C3 plants because of their distinct $\delta^{13}\text{C}/\delta^{15}\text{N}$ ratios (See Fig. 5a).

Statistical analysis

Stable isotope analyses were performed by Japan Certification Services, Inc. (Kanagawa, Japan). The raw data obtained were analysed using the software package 'Stable Isotope Analysis in R' (SIAR) ver. 4.1.1, which estimates the relative contribution of each food source according to a Bayesian approach on the platform of the statistical program R ver. 2.13.1 (Rutz et al. 2010).

RESULTS

The three species of tits differed in their risk sensitivity. Varied tits showed risk-averse choices, whereas marsh tits and great tits showed risk-prone choices at the population level (Fig.2). Risk proneness in the choice-test was weakly sensitive to the food deprivation time prior to tests (min) in a manner independent on the body weight (Fig.3). Differences in risk sensitivity were positively correlated with behavioural granivory, as indicated by food preference tests in the laboratory (Fig.4), Difference in food exploitation was also confirmed by stable isotope analysis of blood samples from wild tits (Fig.5).

Experiment 1: Risk sensitivity

Figure 2 around here

Behavioural data

Behavioural examinations were made after 2–19 months of captivity in laboratory conditions (see Table A1 for list of subjects). Probability-based choices are compared in Fig. 2a–c, in which the reward amount was the same (three pieces of mealworm, or 3A). In choices between a low-probability ($P = 1/3$) and a no risk ($P = 1$) option (a), tits consistently avoided the low probability option. Similar choices were found between a low-probability ($P = 1/3$) vs. a high-probability ($P = 2/3$) option (b), and also between a high-probability ($P = 2/3$) and a no risk ($P = 1$) option, but with a lesser degree (c). On the other hand, in choices between a large amount (3A) and a small amount option (A), tits consistently preferred the larger option (d).

When the amount and the probability contradicted one another (i.e. in which one

reward was better than the other in amount but worse in probability; e.g. 3A(2/3) vs. A(1) in Fig. 2e), tits chose the option with the higher expected value (amount \times probability). However, choices differed among the three species when the expected value was identical between the reward options (3A(1/3) vs. A(1) in Fig. 2f). Varied tits preferred the non-risky option, whereas great and marsh tits preferred the larger, but risky, option. We therefore adopted hierarchical Bayesian model analysis to estimate how tits valued the risky reward (see below). Nonetheless, the transitivity of choices was not violated at the level population (mean values) , thus models based on subjective values could be adopted (see the formulas (2) and (3)).

The observed difference in risk sensitivity could be ascribed to artificial factors such as housing/feeding conditions, rather than to the innate nature of each species, because the period of captivity (i.e., the time from captivity to behavioural tests) varied considerably among subject tits (see Appendix Table A1). In particular, marsh tits and varied tits were tested after 3–19 months of captivity, after being subjected to other behavioural tests (data not shown), whereas great tits were tested earlier, after 2–5 months. Thus whether the period of captivity could influence risk sensitivity was examined using generalized linear mixed model (GLMM) analysis (Table 1).

Table 1 around here

The best model was developed for each pair of options. For choices between 3A(1/3) vs. 3A(1), for example, the best model included only Species for which Akaike's Information Criterion (AIC) was the smallest (125.0) among the other possible models in the set. These models based on other variables (Sex, Captivity period, Month

of the year) showed relatively low level of empirical support given the set of models. For the other 5 pairs of choices, except for that between 3A(1) vs. A(1) (i.e., choices based on amount but not probabilities), Species was involved in the best model. The Captivity period variable was not involved in any pairs, suggesting that the period of captivity had no influence given the set of data. The variables Sex and Month of the year appeared in some pairs but were weaker than Species, and thus were assumed to have little influence.

Estimation of cognitive parameters by hierarchical Bayesian model

Using all of the data obtained in Fig. 2a-f, the subjective values for each of the two options 3A(1/3) and 3A(2/3) were estimated in terms of the probability density calculated using the MCMC method (Fig. 2g and h, respectively). In these graphs, the x-axis denotes the sample frequency (indicative of the probability density; also see the short raster bars), and the y-axis denotes the subjective value of the risky option measured by the number of mealworm pieces. The horizontal lines at 1 (g) and 2 (h) denote the neutral level, around which the samples are expected to distribute if the subjects were risk-neutral and their decisions were based solely on the expected value. In both $P = 1/3$ (g) and $2/3$ (h), varied tits (V) devalued the risky options more strongly than great tits (G) and marsh tits (M) as indicated by their respective medians (arrows).

These distributions were then fitted at the three points of P ($= 1, 2/3, 1/3$) using quadratic curves by estimating the coefficients (Fig. 2i). Great and marsh tits were above the neutral line regardless of the probability, meaning that they are consistently

risk-prone. On the other hand, varied tits stayed below the neutral line at $P = 2/3$, meaning that they are risk-averse. At $P = 1/3$, however, varied tits were almost on the neutral line, indicating that their risk sensitivity is highly skewed. In order to check appropriateness of the assumptions in the developed Bayesian model, we simulated choice data from the fitted posterior distributions. Computational data generated after 1,000 repetitions resulted in a good fit with the observed choice data (see Appendix Figure (a-f)).

Effects of body weight and deprivation time prior to test on choices

The widely accepted energy budget rule might explain the observed divergence in risk sensitivity, even though all of the subjects were freely fed in the housing cages before and after the behavioural tests, so that the daily energy budget was supposed to be positive. It could be argued that the experienced (subjective) deprivation level was not identical among the three species, since the body weight was different. The energy budget may matter at the shorter time scale of hours (rather than the assumed daily budget), so that smaller tits might have suffered more than the larger tits even after identical deprivation, and their preference to risky food temporally differed in the tests. We therefore made a series of *post hoc* analyses to examine whether the individual body weight (g) and/or the deprivation time prior to test (min) could influence the choice data particularly in the condition of identical expected value, namely choices between 3A(1/3) vs A(1) (Fig.2f).

The choices were plotted against body weight (Fig.3a); individual tits were

replicated 4 times, with each individual corresponding to one test trial.. The mean body weight ranged around 18g in varied tits, whereas it was ca.12g in marsh tits and ca.14g in great tits without overlapping among these 3 species. Similar differences in body weights are reported by Jabłoński & Lee (2002) and Park et al. (2005). Our results showed that varied tits were actually heavier and showed a smaller number of choices in the test, indicative of a low degree of risk proneness. However, while great tits were heavier than marsh tits they showed a slightly higher degree of risk proneness (Fig. 2f, g-i), thus making the correlation inconsistent. The choices were also plotted against the deprivation time (Fig. 3b), but the influence on choices was not clearly seen (see below for statistical analyses).

Figure 3 and Table 2 around here

Due to the apparent over-dispersion of the data, we analysed the effects of body weight, deprivation time and their interaction by using GLMMs (Table2). The deprivation time were compared among the 3 species by using ANOVA, but no significant difference was found (d.o.f.=2, F-value = 1.0283, Pr(>F) = 0.363). We therefore developed models on the assumption that tits were tested in the same range of deprivation time.

AICs were compared among all possible models that included deprivation time (deprivation, coefficient α_1), body weight (body, α_2) and their product (Deprivation : Body interaction, α_3), and the best model (AIC = 204.0) proved to be composed solely of deprivation as the explanatory variable. Note that the second best model (AIC = 204.8) included body, but the coefficient α_2 was not significant. All the other models

that included the interaction term showed larger AICs, and α_3 was always not significant. The addition of the Species variable further reduced AICs, such as “Deprivation + Species” (201.8) and “Species + Deprivation : Species” (196.6) , below the “Species” only model (204.5, see Table 1). In contrast, the AIC of the model “Deprivation + Deprivation : Species” had a larger AIC (205.8) suggesting that the inter-species difference predominated over the effects of deprivation time in this study.

Taking all these quantitative statistical analyses into account, we conclude that the heavier tits do not necessarily show higher risk proneness. Whereas, the longer deprivation time tended to lead to higher risk proneness, the effects of the deprivation time did not include the body weight as significant factor. A negative energy budget may partly explain the risk proneness in tits, but this does not account for the differences among three species of tits observed in the present experiment. Other factors such as ecological niche differentiation should be considered.

Behavioural granivory and food preference

Figure 4 around here

Experiment 2: Behavioural data

Behavioural examinations were made after 4–23 months of captivity (Appendix Table A1), namely, after tits had been examined for risk sensitivity. Fig. 4a shows the variation in the number of worm pieces (A) plotted against the number of blocks in the titration procedures; symbols and bars denotes the mean and the standard deviations. The larger the number, the higher the subjective value of one sunflower seed measured

in terms of the number of worm pieces.

In order to examine the correlation between risk sensitivity and the food preference at an individual level, the MCMC method was applied to the Bayesian model to estimate the gradient (Gradient) in terms of the probability distribution (Fig. 4b; also see the formula (6) in Methods). The estimated distribution had a sharp unimodal peak around 3.0–4.5 and never took a value ≤ 0 , indicating that a positive correlation reliably appeared. In Fig. 4c, the subjective value of one sunflower seed (SF) was estimated for each individual in terms of the probability distribution, and the median subjective value was plotted against the median individual discount intensity at low probability ($P = 1/3$; Intensity_{*i, s*}). Using the median of the Gradient distribution (indicated by the downward arrow in Fig. 4b), a dashed line was drawn to represent the regression line.

There was no significant positive correlation when the MCMC computation was run separately for each species (see Appendix Figure (g-h)). In contrast to figure 4b, the posterior density profiles were estimated around Gradient = 0, with the null hypothesis showing that the *gradient* was > 0 , implying that we could not reject this hypothesis in all species. Therefore, the observed non-zero Gradient (or positive correlation) should have been driven by species differences rather than intra-species differences.

Stable isotope analysis of the wild tits blood samples and the candidate food items

Figure 5 around here

The three species of wild tits (circles) differed in $\delta^{15}\text{N}$ contents, but not in $\delta^{13}\text{C}$ (Fig. 5a). For $\delta^{15}\text{N}$ content, great tits and marsh tits were distributed at a higher range,

suggesting that they were more insectivorous than varied tits (Fig. 5b-d; Bayesian analysis). Conversely, the three tit species reared under laboratory conditions (squares) did not differ for either $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$. The isotope contents of laboratory food (rhombus) were much lower. The trophic enrichment factors were estimated as 3.95 ± 0.28 ‰ for $\delta^{15}\text{N}$, and 4.55 ± 0.80 ‰ for $\delta^{13}\text{C}$. Patterns found for candidate food items (circles; means with bidirectional SD) were consistent with those reported previously (Minagawa 1992; Mizukami et al. 2005; Rutz et al. 2010). Animals (invertebrates) contained higher $\delta^{15}\text{N}$ and slightly higher $\delta^{13}\text{C}$ than C3 plants. C4 plants contained a distinctly higher $\delta^{13}\text{C}$ compared with other foods.

SIAR analysis revealed the differences in food exploitation among the three species (Fig. 5b). In this analysis, the relative contribution of food sources to the diet menu was estimated. When the peaks of the estimated distributions were compared among different food categories (invertebrates, C3 and C4), we assumed that varied tits were more granivorous than great tits and marsh tits. It is also plausible that C4 plants marginally contributed to the diet of any tit species.

DISCUSSION

The three sympatric species of tits differed in their risk sensitivity (Fig. 2), in a manner correlated with their respective patterns of diet menu; the more granivorous varied tits were risk averse, whereas the more insectivorous marsh tits and great tits were unusually risk prone. Deprivation time, thus a transient negative energy budget during the test, may partly explain the risk proneness, but the difference among species

could not be attributed to the difference in body weight (Fig. 3). In the present study, the diet menus were examined using two methods, namely, food preference determined using binary choice tests (Fig. 4), and food exploitation determined using isotope analysis (Fig.5) which confirmed the results of a previous behavioural study (Nakamura 1970). Since risk sensitivity was measured in laboratory conditions at the individual level using binary choice tests, a comparable measure of diet menu at the individual level was performed in the form of food preference (Fig. 4) that reflected food exploitation estimated for wild tits (Fig. 5). It can thus be concluded that the measured food preference honestly represented aspects of the pattern of food exploitation.

Does the measured risk sensitivity reflect that in the wild?

Surprisingly few studies have been done on risk sensitivity in the wild. Carter & Dill (1990) and Carter (1991) are exceptionally successful cases in which results from captivity (Carter & Dill 1990) and from the wild (Carter 1991) were compared for bumble bees, showing identical patterns of risk sensitivity in both conditions at the population level. In this study, the captivity period as well as the month of the year when the behavioural tests were carried out, did not contribute to the choices in experiment 1 (Table 1). This suggests that risk sensitivity is one of the behavioural traits that is not influenced by captivity and can be assumed as a personality or behavioural syndrome (Herborn et al. 2010). It should be noticed that tits showed a higher risk proneness at tests when they had been deprived of food for a longer period (Fig.3 and Table 2); risk sensitivity can be modified depending on contexts. On the other hand,

divergent risk sensitivity might be caused by some other unspecified artificial factor that tits experienced during captivity. In this context, we should notice that the experiment 1 was performed using mealworms, which was the preferred food particularly in marsh tits and great tits as shown in Experiment 2. If tits were instead tested using sunflower seed that is more preferred in varied tits, different patterns of risk sensitive choices could arise. Actually, Craft et al. (2011) reported risk aversion (or fewer choices for a variable option) in rats only when high caloric sugar pellets were used, although indifferent choices occurred when tested using low caloric (and less preferred) grain pellets. The pattern in rats was therefore contrary to that found in our present study.

Alternatively, different risk sensitivities might arise, if tits were tested using larger food items (e.g., larger pieces of mealworms) rather than the seeds. As pointed out by Wright and Radford (2010), risk-sensitive (or variance-sensitive) choices may differentiate based on the degree of convexity of the value function. Particularly in great tits and marsh tits, concavity could have occurred in the lower range of food amount instead (also see below on the issue of dominance). Further studies are required.

Is the divergent risk sensitivity relevant to food storing behaviour?

The observed difference in risk sensitivity cannot be explained by differences in food storing behaviours. Since the capability to store food could influence the energy budget by directly reducing the probability of starvation, food storers may be more risk averse, whereas non-storers may be more risk prone. Food storing black-capped chickadees are reported to show a clear risk aversion (Barkan 1990). However, in this

study the food storing marsh tits (Stevens & Krebs 1986) were risk prone, whereas the similarly storing varied tits (Higuchi 1975) were risk averse. It is however to be noticed that, in the present study, tits were given abundant amount of food in the cage, and tits did not show food cache behaviours in captivity. If tested under a daily negative energy budget, in which the storing birds depended more on the food cache, tits could reveal different patterns of risk sensitivity. Further experimental studies are required also on this point.

Evolutionary scenarios potentially underlying the divergent risk sensitivity

The present results suggest that risk sensitivity could flexibly change even within a taxon. Though marsh tits are closer to varied tits (Gill et al. 2005), they showed risk sensitivity similar to great tits that are taxonomically more distant. Risk sensitivity may thus be subject to changes under local selection pressure, which varies uniquely with respective bio-geography. We might assume that tits differentiated their respective niche such as diet menu, which then caused the divergent risk sensitivity among the sympatric tits in the local Hokkaido area, through yet unidentified mechanisms. Or otherwise, risk-sensitivity might primarily diversified, leading to secondarily diversified diet menus among tits, as has been argued for in the sex differences in the green woodhoopoes (Wright and Radford, 2010). We should also note that it remained unanswered as to whether collecting seeds is actually un-risky whereas hunting for insects is more risky specifically in Hokkaido. It will be critically important to examine if the insects foods could be distributed in a manner different from seed foods

particularly in winter.

Alternatively, direct inter-species interactions may matter. In a field study in England, where the larger varied tits are absent, Stephens & Ydenberg (cited in Stephens 1982) reported that great tits were risk averse. In our present study, on the other hand, tits are sympatric and form inter-species foraging flocks particularly during harsh winters (Nakamura 1967; Jabłoński & Lee 1998, 2002). It is possible that physically dominating varied tits may drive other two species towards the less adaptive niche in which they depend more on risky food items. However, we are unable to explain why the great tits had a higher risk-proneness than the lighter marsh tits (Fig.2), even though the former was heavier than the latter (Fig. 3a). If the body weight uniquely determined the foraging dominance, the divergent risk sensitivity cannot directly ascribed to the dominance hierarchy in the flock foraging. Possible functional link between the social dominance (and accompanying aggressiveness) with the risk sensitivity should be searched for in those animals foraging in the wild.

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FIGURE LEGENDS

Figure 1 Binary choice apparatus.

(a) Two food trays, each with a thin plastic cover, were placed under a transparent acrylic cover. Cued by the colour pattern on the cover, birds pulled the string attached to one of these trays. Choosing one tray resulted in the other being automatically withdrawn, and the stopper bars dropped. The bird then removed the cover of the chosen tray and gained the food inside. (b) Association between the colour patterns and the food rewards for studying the risk sensitivity. The colour pattern reflected the amount (1 or 3 pieces of freshly cut mealworm) and probability ($P = 1, 2/3$ or $1/3$). For example, the tray 3A(1) was covered in pink/green, and contained 3 pieces at $P = 1$. For these 4 rewarding options, all possible combinations were tested using binary choices, namely in 6 pairs (4C_2). (c) Association between the colour patterns and the food rewards for studying the food preference in behavioural titrations. Birds chose between one sunflower seed and A pieces of mealworm, where A was adjusted towards an equal choice ratio.

Figure 2 Distinct risk preference based on behavioural data.

(a-f) For each species, 6 subject birds were tested for 6 pairs of choices. Symbols denote the average choice value over 4 replicate test blocks for each subject, and * indicates the mean for each species. Horizontal dashed lines indicate even choices. (g, h) The estimated subjective values of risky food options are plotted as raster (MCMC samples), and the probability density distributions are shown by curves. The probability of risky

options was 1/3 (**g**) and 2/3 (**h**). Neutral lines are drawn at the expected value, i.e., value = amount \times probability = 1 (**g**) and =2 (**h**), respectively. Arrows indicate the median for each species. (i) A fitted curve is drawn against the probability of risky options. Since no discount occurs for the constant option, the value is equal to the amount (= 3) at $P = 1$. Birds are risk prone above the neutral line, while they are risk averse below the line.

Figure 3 Effects of body weight and deprivation time on the risk proneness revealed in the binary choices between options of identical expected values.

(**a**) The numbers of choice of risky option (i.e., 3A(1/3)) among 12 test trials are plotted against the body weight (g) of the individual subject tit. Single symbols denote single test trials, and data obtained from one individual are represented four times. (**b**) The same set of data is plotted against the deprivation time (min) prior to the start of test trials. The dashed line is drawn based on the best model chosen for the smallest AIC (204.0; see Table 2), which included Deprivation as sole meaningful variance ($X = -0.80 + 0.0085 * \text{deprivation}$).

Figure 4 Distinct food preference based on behavioural data.

(**a**) Numbers of the worm pieces are plotted against the number of blocks. Symbols denote mean \pm s.e.m. for each species (varied and marsh tits: $n=6$, great tits: $n=5$). (**b**) Estimated gradient is plotted as raster (MCMC samples) and the probability density distribution is shown by a single-peaked curve. The downward arrow indicates the median. (**c**) Correlation plots of estimated intensity and subjective value of a seed.

Intensity is major indicator of risk sensitivity in our model. The dashed line is a regression line drawn using the estimated values of gradient and intercept.

Figure 5 Distinct food exploitation based on metabolism data.

(a) Stable isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the blood samples obtained from wild birds (\circ ; varied tits: $n = 5$, marsh tits: $n = 4$, great tits: $n = 9$). Data obtained from birds fed with laboratory food for longer than 1 year (\square ; varied and marsh tits: $n=4$, great tits: $n=3$) are superimposed; notice that the variances are much smaller for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and that the ratio of isotopes was influenced by the diet. Possible food items were also sampled from the field and grouped into 3 categories, i.e., invertebrates (16 species), C3 plants (7 species), C4 plants (3 species). Symbols denote mean \pm SD. **(b-d)** Estimations of the proportions of each food category exploited by each of the three species. The vertical dashed lines denote the peaks of invertebrates and C3 plants for varied tits.

Appendix Figure

(a-f) Simulated choice data generated from the fitted posterior predictive distributions obtained by the hierarchical Bayesian model analysis. Note that the computed data (circles) well fit with the observed means (asterisks; the same set of data shown in Fig. 2a-f). Horizontal dashed lines indicate even choices. In the hierarchical Bayesian model developed in this study, MCMC computation gave rise to frequency distribution for each of the parameters involved in the formulae (2), (3) and (4) (see

Statistical analysis in Methods). Based on these distributions, means of individual tits (circles) and their 95% confidence intervals (bars) were calculated as follows. (i) Single value was sampled from the 2.5-97.5% interval of the frequency distribution for each parameter. (ii) The set of sampled values were substituted to the formulae (1) to (4), yielding a single value of choice probability $Q(X)$. (iii) The processes (i) and (ii) were repeated 1,000 times. (iv) Of these 1,000 sampling-based values of choice probability, individual means and their 95% confidence intervals were determined.

(g-h) Lack of significant correlation between discount intensity and the value of seed in each species. Estimated posterior distribution of the gradient were shown for each species (left) and the linear regressions drawn on the basis of the estimated gradients (dashed lines) were shown in each species, in a manner superimposed on the data plot shown in Figure 4(c). Results of a hierarchical Bayesian model analysis. In order to examine if significant inter-individual correlation occurred in each species, we assumed three species-specific gradients (one for each species) as prior distributions. Because each sample contained only a few individuals ($n = 5-6$), we were unable to reliably estimate both the intercept and the gradient by using MCMC computation. In order to circumvent the problem, we made an additional assumption (constraint) such that the mean of the prior distribution of the intercept is equal to the estimated “value of a seed” for each species. With this assumption, MCMC computation gave rise to reliable estimates on the gradient of the linear regression (coefficient of correlation) in each species. We thereafter calculated the probability (p) that the computed posterior distribution of the gradient included 0 (zero). Actually, in all tit species, the p turned out

to be larger than 0.05, thus indicating that the null hypothesis (no correlation) should not be discarded.

Table 1

Generalized linear mixed models (GLMMs) reveal critical parameters involved in choices

Choice tests	The best model		Full model	Null model
	Model	AIC	AIC	AIC
3A(1/3) vs. 3A(1)	Species	125.0	129.1	135.3
3A(1/3) vs. 3A(2/3)	Species + Sex	119.9	121.6	133.7
3A(2/3) vs. 3A(1)	Species + Month of the year	145.7	148.8	150.1
3A(1) vs. A(1)	Null	165.2	170.6	165.2
3A(2/3) vs. A(1)	Species	186.8	192.5	192.1
3A(1/3) vs. A(1)	Species	204.5	207.9	206.4

The influences of the four explanatory variables (Species, Sex, Captivity period, Month of the year) were estimated in terms of the Akaike Information Criteria (AICs). For each type of choice tests, models were exhaustively searched for toward the best model that yielded the smallest AIC. For example, the model denoted as “Species” contains only the variable Species. The Full model contained all of the four variables (i.e., Species + Sex + Captivity period + Month of the year). The Null model contained no variables but intercepts. Choices (the response variable) were assumed to follow a binomial distribution with an individual random effect.

Table 2

GLMMs for estimating the effects of body weight and food deprivation time on the risk sensitivity in the choice test between 3A(1/3) vs A(1)

Models	AICs	α_0 (intercept)	α_1 (Deprivation)	α_2 (Body)	α_3 (Deprivation : Body)
$X = \alpha_0$ (null model)	206.4	0.44	-	-	-
$X = \alpha_0 + \alpha_1 * \text{Deprivation}$	204.0	-0.80	0.0085 #	-	-
$X = \alpha_0 + \alpha_2 * \text{Body}$	207.2	2.69	-	-0.15	-
$X = \alpha_0 + \alpha_1 * \text{Deprivation} + \alpha_2 * \text{Body}$	204.8	1.52	0.0085 #	-0.16	-
$X = \alpha_0 + \alpha_3 * \text{Deprivation} * \text{Body}$	205.5	-0.58	-	-	0.00048
$X = \alpha_0 + (\alpha_1 + \alpha_3 * \text{Body}) * \text{Deprivation}$	205.0	-0.70	0.020	-	-0.00084
$X = \alpha_0 + (\alpha_2 + \alpha_3 * \text{Deprivation}) * \text{Body}$	204.9	2.80	-	-0.25	0.00060
$X = \alpha_0 + \alpha_1 * \text{Deprivation} + \alpha_2 * \text{Body} + \alpha_3 * \text{Deprivation} * \text{Body}$ (full model)	206.8	1.57	0.0082	-0.16	0.000026

All possible combinations available with additional explanatory variables (Deprivation for deprivation time (min) prior to test, and Body for body weight (g) of individual) are shown for the AICs and the estimated coefficients. For the other variables, see Table 1.

indicates that the estimates could include the value = 0 at low probability < 0.05, thus contribution of these terms is supposed to be significant.

Appendix Table A1

List of subject tits

ID	Sex	Capture	Captive period before EXP1 (month)	Exp1-risk sensitivity	Exp2–food preference	Stable isotope analysis
GT(black)	M	Feb 2010	3	May-Jul 2010	died on Sep 2010	○
GT(orange)	F	Feb 2010	3	May-Jul 2010	Oct-Dec 2010	○
MT(white)	M	Mar 2009	14	May-Jul 2010	Oct-Dec 2010	
MT(blue)	M	Mar 2009	14	May-Jul 2010	Oct-Dec 2010	
VT(lightblue)	M	Dec 2008	17	May-Jul 2010	Oct-Dec 2010	
VT(lightgreen)	F	Dec 2008	17	May-Jul 2010	Oct-Dec 2010	
GT(gray)	M	Feb 2010	5	Jul-Sep 2010	Oct-Dec 2010	○
GT(lightgreen)	F	Feb 2010	5	Jul-Sep 2010	Oct-Dec 2010	○
MT(gray)	M	Mar 2009	16	Jul-Sep 2010	Oct-Dec 2010	
MT(yellow)	M	Mar 2009	16	Jul-Sep 2010	Oct-Dec 2010	
VT(purple)	F	Dec 2008	19	Jul-Sep 2010	Oct-Dec 2010	
VT(red)	F	Mar 2009	16	Jul-Sep 2010	Oct-Dec 2010	
GT(lightblue)	M	Feb 2011	2	Apr-May 2011	Jun 2011	○
GT(yellow)	F	Jan 2011	3	Apr-May 2011	Jun 2011	○
MT(pink)	F	Dec 2010	4	Apr-May 2011	Jun 2011	○
MT(red)	F	Dec 2010	4	Apr-May 2011	Jun 2011	○
VT(gray)	M	Jan 2011	3	Apr-May 2011	Jun 2011	○
VT(black)	M	Jan 2011	3	Apr-May 2011	Jun 2011	○
GT(purple)	M	Dec 2010				○
GT(blue)	M	Mar 2011				○
GT(white)	F	Mar 2011				○
MT(orange)	M	Dec 2010				○
MT(green)	F	Mar 2011				○
VT(orange)	F	Dec 2010				○
VT(white)	F	Dec 2010				○

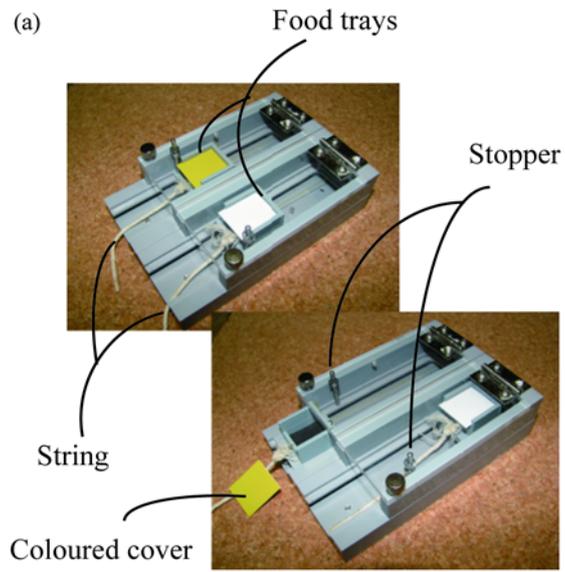
ID (species and colour of leg ring, GT: great tit, MT: marsh tit, VT: varied tit), Sex (Male or Female), month and year of capture, capture period of captivity before EXP 1, periods of EXP 1 and 2, and usage for stable isotope analysis are shown for each individual. * Sex was not identified because of insufficient blood sample.

Appendix Table A2

List of potential food items collected in the capture site for stable isotope analysis

	Family	Order	Class
Invertebrate			
Insect	Dermestidae: adult	Coleoptera	Insecta
	Dermestidae: larva		
	Chrysomelidae		
	Carabidae (2 spp.)		
	Elateridae		
	Aphidoidea	Hemiptera	
	Pentatomidae		
	Lygaeidae		
	Sciomyzidae	Diptera	
	Chironomidae		
Spider	Chrysopidae	Neuroptera	Arachnida
	Thomisidae	Araneae	
	Tetragnathidae		
Earthworm	Antrodiaetidae		Oligochaeta
	Megascolecidae	Haplotaxida	
	Bradybaenidae	Pulmonata	
Snail			Orthogastropoda
Plant			
C3 plant	Fabaceae	Fabales	Magnoliopsida
	Lamiaceae	Lamiales	
	Asteraceae	Asterales	
	Cannabaceae	Urticales	

	Pinaceae	Pinales	
	Grossulariaceae	Rosales	
	Rosaceae		
C4 plant	Poaceae (3 spp.)	Poales	Liliopsida



(b)

	Colour	Amount	Probability
A(1)	 Yellow	1	1
3A(1)	 Pink/ Green	3	1
3A(2/3)	 Red/ Blue	3	2/3
3A(1/3)	 Orange/ Brown	3	1/3
S-	 White	0	-

(c)

	Colour	Amount	Probability
Seed	 Orange/ Purple	1	1
Worms	 Yellow/ Green	A	1
S-	 White	0	-

Figure 1.

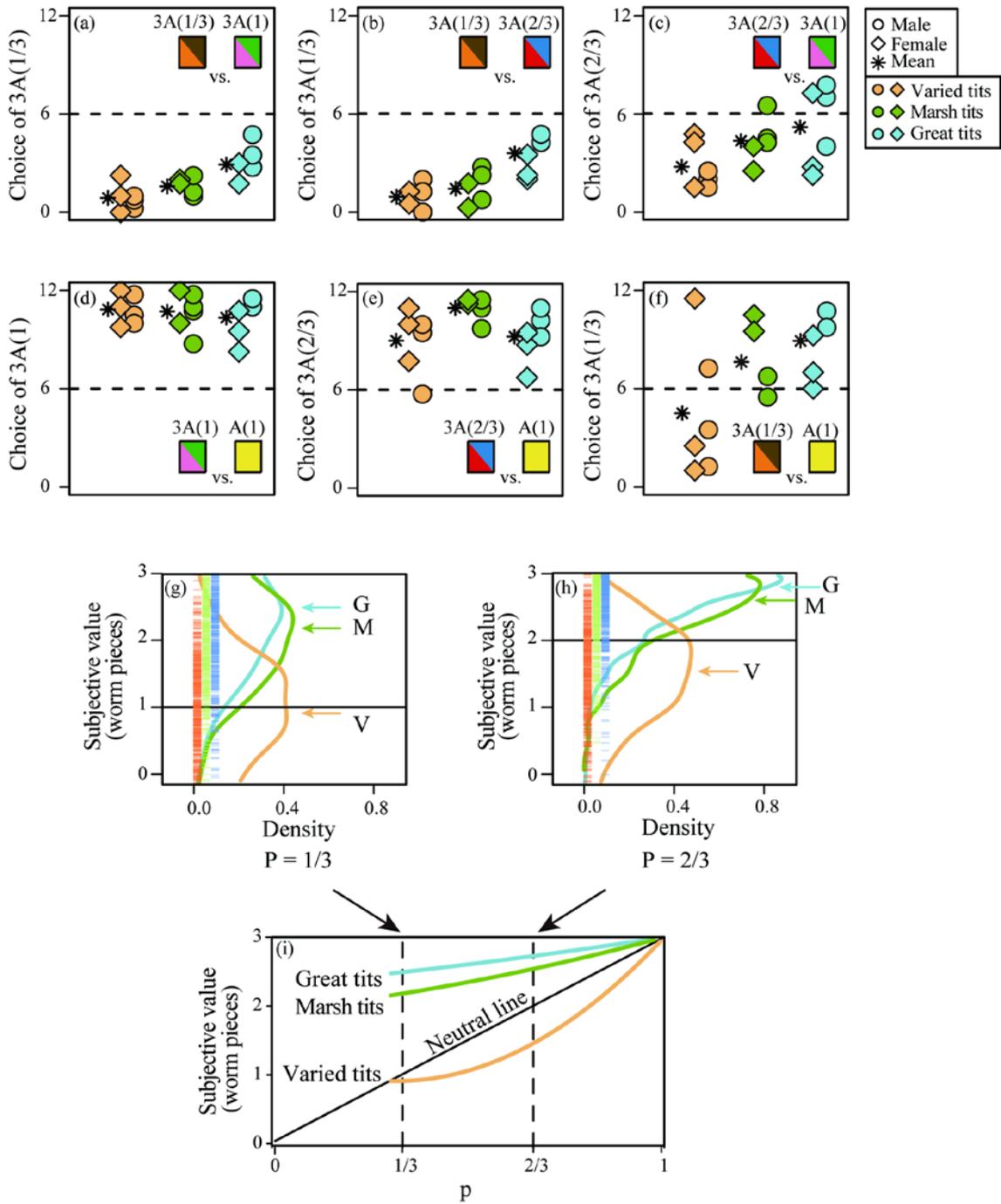


Figure 2.

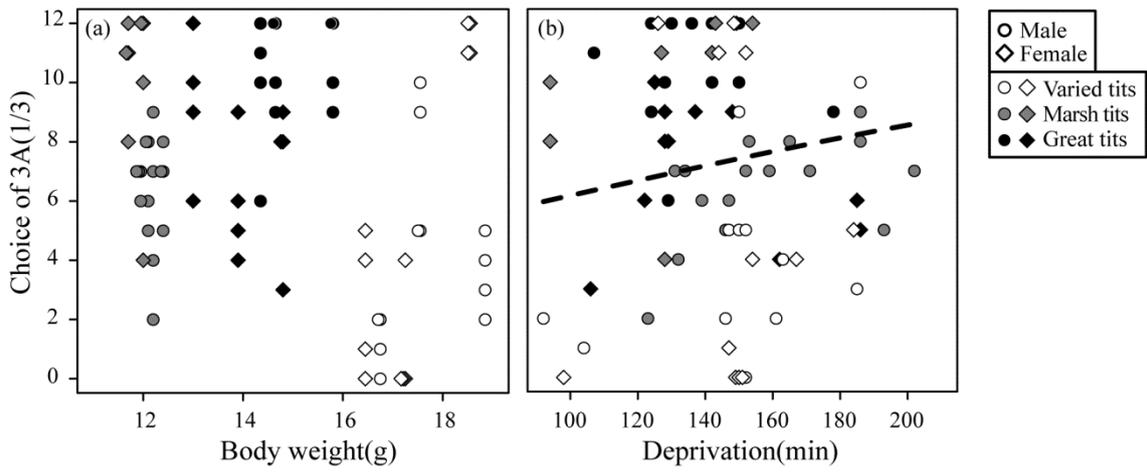


Figure 3.

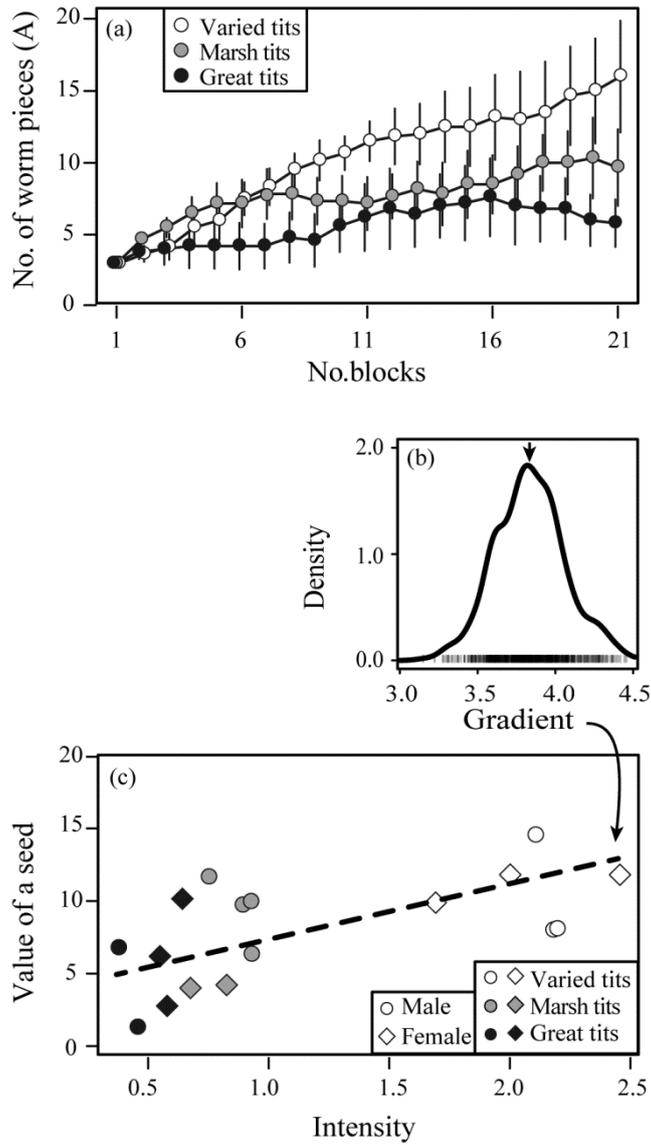


Figure 4.

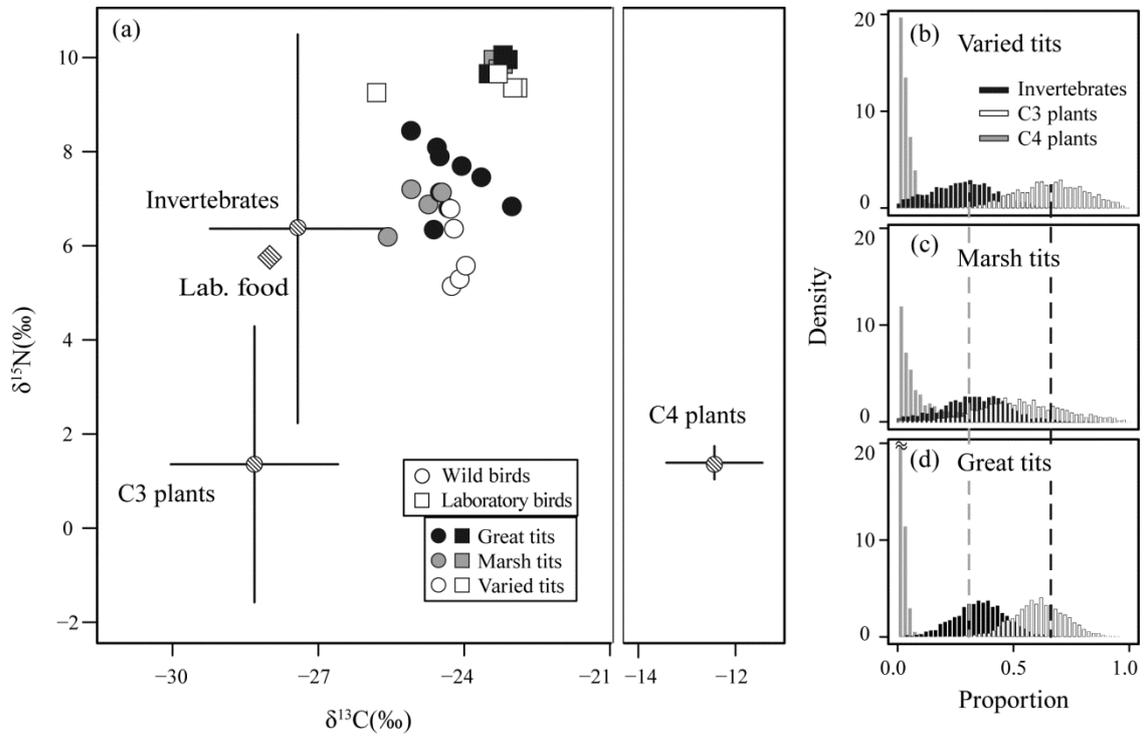
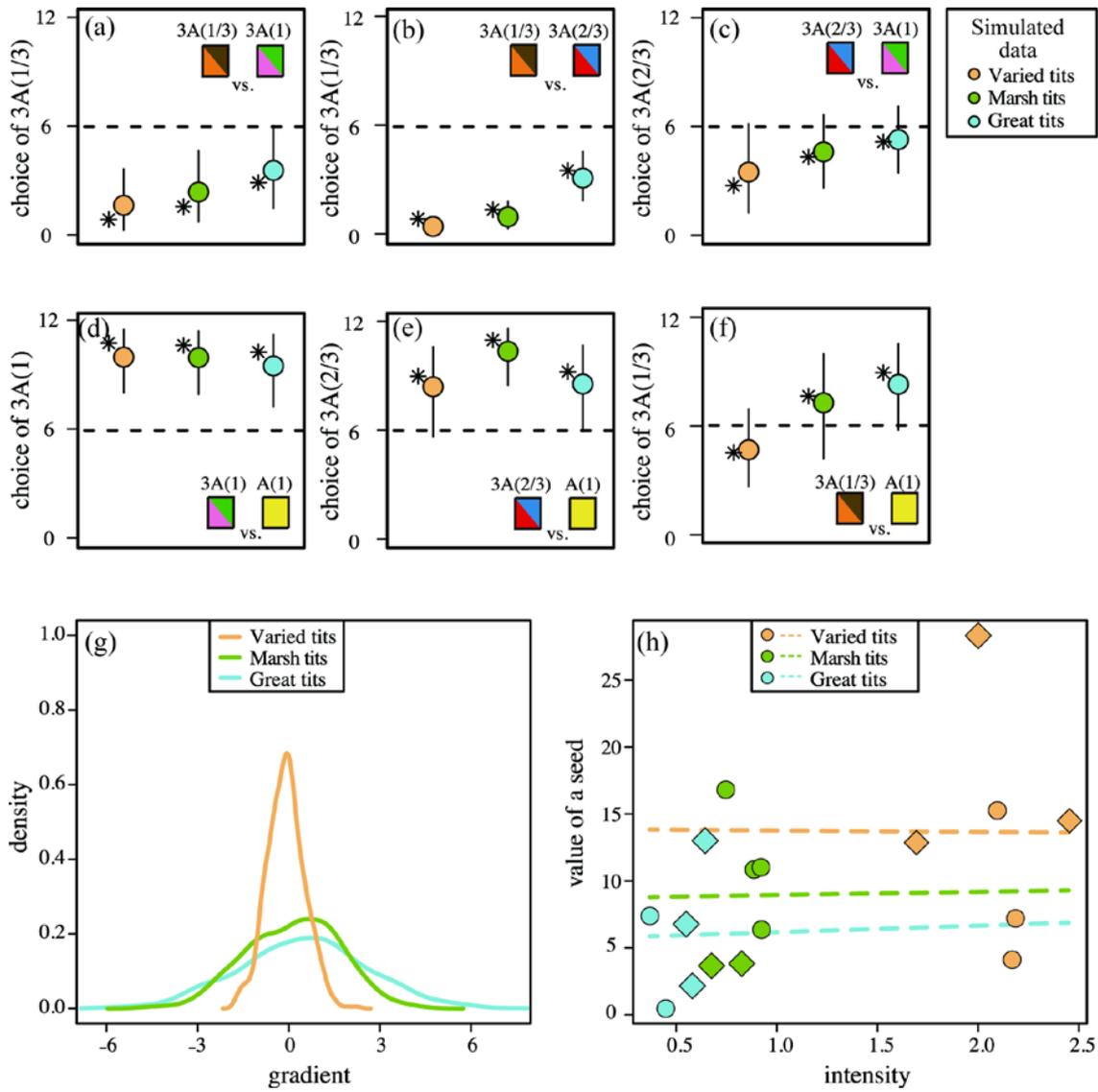


Figure 5.



Appendix figure.