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Dietary competition between the alien Asian Musk Shrew (*Suncus murinus*) and a reintroduced population of Telfair's Skink (*Leiolopisma telfairii*)

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Running title: Niche overlap - alien vs. native predators

Abstract

Reintroduction of rare species to parts of their historical range is becoming increasingly important as a conservation strategy. Telfair's Skinks (*Leiolopisma telfairii*), once widespread on Mauritius, were until recently found only on Round Island. There it is vulnerable to stochastic events, including the introduction of alien predators that may either prey upon it or compete for food resources. Consequently skinks have been introduced to Ile aux Aigrettes, another small Mauritian island that has been cleared of rats. However, the island has been invaded by Asian Musk Shrews (*Suncus murinus*), a commensal species spread by man well beyond its natural Asian range. Our aim was to use next generation sequencing to analyse the diets of the shrews and skinks to look for niche competition. DNA was extracted from skink faeces and from the stomach contents of shrews. Application of shrew and skink-specific primers revealed no mutual predation. The DNA was then amplified using general invertebrate primers with tags to identify individual predators, then sequenced by 454 pyrosequencing. 119 prey MOTUs (molecular taxonomic units) were isolated, though none could be identified to species. Seeding of cladograms with known sequences allowed higher taxonomic assignments in some cases. Although most MOTUs were not shared by shrews and skinks, Pianka's niche overlap test showed significant prey overlap, suggesting potentially strong competition where food resources are limited. These results suggest that removal of the shrews from the island should remain a priority.

Introduction

The introduction of locally extinct species to suitable habitats within their wider geographical range is an increasingly important component of conservation strategies (Seddon *et al*. 2012). When the distribution of a threatened native species has contracted to one or a few isolated sites it is highly vulnerable to stochastic events, such as the introduction of alien species, which could rapidly destroy a last remaining stronghold. Translocation of such a species to a new habitat becomes a conservation priority. The habitat of such an alternative refuge should ideally be free of threats from alien species, providing ecological conditions suitable for reintroductions. However, removal of alien species can often be physically impossible (for example with many invertebrate species) or prohibitively expensive. In some cases the effective techniques for removal of an alien need to be developed. Under such conditions it may be necessary to attempt reintroductions under less than ideal conditions and pragmatically determine whether a rare species can thrive in sympatry with remaining aliens. Examples of successful translocations are birds such as the Kakapo (*Strigops habroptilus*) between offshore islands in New Zealand (Elliott *et al*. 2001), and both pink pigeon (*Columba mayeri*) and Mauritius Fody (*Foudia rubra*) to Ile aux Aigrettes (Seymour *et al*. 2005; Cristinnace *et al*. 2009), and reptiles including whiptail lizards (*Cnemidophorus vanzoi*) to Praslin Island, Saint Lucia (Dickinson & Fa 2000), Antiguan racers (*Alsophis antiguae*) to offshore islands of Antigua (Daltry *et al*. 2001) and lizards to New Zealand islands (Towns & Ferreria 2001).

Asian Musk Shrews, *Suncus murinus* (Soricidae), are a highly invasive species spread by man to numerous locations outside its natural Asian range (Ruedi *et al.* 1996). It is a commensal species with man, often living in and around houses and spread by us between land masses. It was introduced to Mauritius in the 18th century and has been implicated in the loss of endemic vertebrate and invertebrate species there (Jones 1993; Cole *et al*. 2005; Cheke & Hulme 2008; Solow *et al*. 2008) as well as in other parts of the world, such as Guam (Fritts & Rodda 1998). Between 2009 and 2010, the shrew invaded Flat Island to the north of Mauritius, leading to the localised loss of three endemic reptile species within 18 months (N Cole unpublished data). It is thought to have been introduced to Ile aux Aigrettes (southeast of Mauritius) in the early 20th century where it spread rapidly (Cheke & Hume 2008). Seymour *et al*. (2005) calculated that 20 females of *S. murinus* on Ile aux Aigrettes could potentially generate a population of 550 individuals over a five month reproductive season. On Ile aux Aigrettes, eradication programmes appeared to be successful for a while, but it soon became clear that some individuals had survived and population recovery was rapid (Varnham *et al*. 2002; Seymour *et al*. 2005; Solow *et al*. 2008). Cats (*Felis catus*) and brown rats (*Rattus rattus*) were successfully eliminated from Ile aux Aigrettes by 1991 as part of a habitat restoration programme (Jones & Hartley 1995), but this may have simply exacerbated the problem with the alien shrews, releasing them from predation and competition with these equally alien predators.

Telfair’s Skinks (*Leiolopisma telfairii*) are one of eight species of endemic Mauritian reptiles that managed to survive on Round Island, where they are thriving in the absence of alien predators (North *et al*. 1994; Pernetta *et al.* 2005). Historically these skinks lived on mainland Mauritius and on a number of surrounding islands (Cheke & Hume 2008). As an insurance against loss of the Round Island population, the skinks were introduced to Ile aux Aigrettes between 2006 and 2010 where the adults are surviving well, but there is strong evidence that juveniles may be directly preyed on by Asian Musk Shrews. There is also evidence that adult skinks prey upon shrews and annual population surveys of terrestrial vertebrates along transect lines on Ile aux Aigrettes demonstrated a 68% decline in the relative abundance of shrews since skinks were released (N. Cole unpublished data). However, the skinks and shrews may also be limited by resource competition. Evidence from the eradication programme, based upon live trapping, showed that as numbers of shrews declined, their mean body mass increased considerably. This suggested that food resources were limiting and that this increase in mass was the result of release from intraspecific competition (Seymour *et al.* 2005). It follows that interspecific competition, between shrews and skinks, might also therefore have an adverse effect upon the skinks if they share the same prey. Both shrews and skinks are omnivorous, eating both plant and animal foods, which may buffer them against food shortages during the dry season on Ile aux Aigrettes, when invertebrate prey are scarce (Cole & Harris 2011). Little is known about the invertebrate prey species consumed by shrews and skinks, although morphological identification of fragments of larger prey in faecal samples has provided some information but mainly at higher taxonomic levels (Vinson & Vinson 1969; Pernetta *et al.* 2005; Richards 2007; Zuël 2009; Copsey *et al.* 2011). These studies using morphological examination of faecal samples from skinks, revealed predation on Araneae, Blattaria, Chilopoda, Coleoptera, Collembola, Decapoda, Dermaptera, Diptera, Embioptera, Hemiptera, Homoptera, Hymenoptera, Isopoda, Lepidoptera, Opisthopora, Orthoptera, Pseudoscorpionida, Scorpionidae, Stylommatophora and Thysanura. Less information appears to exist on invertebrates in the diets of Asian Musk Shrews, which are generally considered to be highly omnivorous, incorporating significant quantities of arthropods in their diets including Orthoptera, Hymenoptera, Blattaria and Chilopoda (Advani & Rana 1981; Prakash & Singh 1999; Lathiya *et al*. 2008). On Ile aux Aigrettes the African land snail *Achatina fulica* was consumed when used as bait in traps (Varnham *et al*. 2002). Given their current wide geographical distribution and adaptability, the shrews are likely to have very different diets within different regions and ecosystems.

The problem with morphological identification of prey remains in the guts or faeces of vertebrates is that it is biased towards prey with hard parts that resist digestion (Symondson 2002). It requires a high level of taxonomic skill and the diagnostic features, essential for species-level identification, may not survive digestive processes (Ingerson-Mahar 2002; Sunderland *et al*. 2005). An alternative approach is to analyse gut and faecal samples using PCR (Symondson 2002; King *et al*. 2008), which can now be combined with next generation sequencing (NGS) (Pompanon *et al.* 2012). General invertebrate primers can potentially amplify all invertebrates consumed, generating DNA 'barcodes' (diagnostic sequences from a defined region of a gene) for each prey species (Pompanon *et al.* 2012). In tropical ecosystems, such as on Ile aux Aigrettes, the invertebrate fauna has not been barcoded and few, if any, taxa are likely to be found on databases such as GenBank or BOLD (Barcoding of Life Database). However, the sequence output from NGS analyses can be clustered into MOTUs (molecular operational taxonomic units) (Floyd *et al.* 2002) as a proxy for species and can be used to analyse dietary overlap between predator species (Razgour *et al.* 2011). Two predator species may, for example, be consuming the same families of invertebrates but completely different species, and the MOTU approach will reveal this, even when the Linnaean identities of those species cannot be determined. We therefore used next generation sequencing to analyse the invertebrate diets of the shrews and skinks, then tested the hypothesis that there was significant niche overlap between the alien and native species, potentially leading to competition. Tests such as Pianka's niche overlap test (Pianka 1973) do not necessarily reveal where the most significant dietary overlaps lie. We therefore further tested the hypothesis that many prey species are eaten occasionally, probably opportunistically, while a smaller number of key prey species are shared and form a potentially significant part of the diet. Only competition for these prey might be limiting for predator populations. We also tested the hypothesis that shrews and skinks may be competing in a more direct way, by preying on one another.

Methods

Predator sampling

Samples were collected over an eight week period from the 10th March to the 5th May 2011, on Ile aux Aigrettes, Mauritius. This 26 ha coralline island nature reserve is leased to, and managed by, the Mauritian Wildlife Foundation. Shrews were initially caught using Sherman traps. However, trapped shrews had very little material in their guts by the time they were removed. Any remaining gut contents often included bait, and shrews were observed to eat ants from the bait, creating false trophic links. Shrews with full stomachs were subsequently caught more successfully by hand and killed (using UK Home Office approved techniques, Animals (Scientific Procedures) Act 1986) during surveys across the island, both in the early morning and late afternoon/early evening. They were brought back immediately to the field station, dissected under sterile conditions to obtain stomach samples, sexed and measured. Gender was confirmed by post mortem examination for the presence or absence of testes. The length from nose to base of tail was measured to the nearest mm. The presence or absence of foetuses was recorded for females. For males it was often possible to determine adult or juvenile status based on the development of the testes. Females were classed as juveniles if they were less than 12g. The stomach was stored in 94% ethanol at -20°C.

Telfair's Skinks were caught by hand and induced to defecate by gently massaging the belly. A sterile tube was placed below the cloaca to catch the faeces, which was topped up with 94% ethanol and kept at -20°C. Animals were sexed using morphological characteristics including hemipenal eversion of males. Each individual was identified from a unique subcutaneous PIT (Passive Integrated Transponder) tag number, which had been implanted during translocation from Round Island. Finally, measurements of snout-vent length (SVL) were taken. For a full list of both shrews and skinks caught and analysed, with measurements, refer to Table S4.

DNA extraction

DNA was extracted from faecal and gut samples using the QIAmp DNA Stool Mini Kit (QIAGEN), according to the manufacturer's instructions. Additionally, DNA was extracted from a range of invertebrate samples collected from Ile aux Aigrettes, along with tissue samples from shrews and skinks, for primer testing, using the DNeasy tissue kit (QIAGEN), according to the manufacturer's instructions.

Primer selection for pyrosequencing

Published universal PCR primers were tested in a number of different combinations for their ability to amplify DNA from 29 different taxonomic groups of invertebrates (19 orders) collected from Ile aux Aigrettes. Temperature gradient PCRs were performed for each primer pair to determine the optimal annealing temperature at which the most taxa would amplify. PCRs were run on a Peltier Thermal Cycler (Bio-Rad Laboratories, CA, USA) using Multiplex PCR kit (Qiagen) under the following conditions: 1X Master Mix, 0.2 μM each primer and 10ng / μL of DNA with an initial denaturation at 95^{o}C for 15 min, 45 cycles of 94^{o}C for 30 s, a gradient of 45–60^{o}C for 90 s and 72^{o}C for 90 s, and a final extension at 72^{o}C for 10 min. DNA of the shrews and skinks were also included so that primer pairs which did not cross-amplify with the predators could be identified. Water was included in each PCR in place of DNA as a negative control. From the large number of primers tested (some unpublished) the best proved to be the forward primer LCO-1490 (Folmer *et al.* 1994) combined with the reverse primer Uni-MiniBar-R (Meusnier *et al.* 2008), which produced a COI (cytochrome oxidase I) amplicon of 177 bp. These primers were found to amplify 28 of the 29 local taxa at an annealing temperature of 49^{o}C and 42 cycles, with no cross-amplification of the predators (Table S1). A second useful primer pair, combining LCO-1490 with ZBJ-ArtR2c (Zeale *et al.* 2011), produce a COI amplicon of 225 bp, and was found to amplify 27 of the 29 taxa at an annealing temperature of 52°C and 40 cycles (Table S1), but in initial tests weakly cross-amplified the shrew. We therefore used the LCO-1490 / Uni-MiniBar-R for further analysis. All other primer combinations tested co-amplified the shrew and/or skink DNA more strongly or amplified a lower range of invertebrate taxa.

Pyrosequencing

LCO-1490 and Uni-MiniBar-R, modified with fusion primers and MIDS (Multiplex Identifiers in the form of unique DNA tags), were used to amplify faecal/gut DNA extracts from shrews and skinks using PCR conditions described above. By using a unique combination of MIDS on both the forward and reverse primers for each individual predator, MOTUs could be assigned to each predator later bioinformatically. DNA from 41 shrew stomach samples and 29 skink faecal samples were successfully amplified. PCR products were run through a 2% agarose gel stained with ethidium bromide and quantified using UVP VisionWorks® LS Analysis software by comparing fluorescence with known concentrations using MassRuler Low Range DNA ladder (Fermentas). Samples were then pooled together in differing proportions to obtain an approximately equal amount of DNA in the final mixed sample. The pooled sample was purified using the QIAquick PCR Purification Kit (QIAGEN) and pooled DNA concentration quantified by Nanodrop ND-1000 Spectrophotometer.

The DNA was sent to the Genepool, Edinburgh, for NGS. This was performed using the Roche 454 GS-FLX (Roche Applied Sciences) emPCR Lib-L method.

Sequence Analysis

Sequences were analysed using the Galaxy platform (https://main.g2.bx.psu.edu/root, Giardine *et al.* 2005; Goecks *et al*. 2010; Blankenberg *et al*. 2010) and Bioedit (T. Hall, http://www. Mbio.ncsu.edu/bioedit/bioedit.html). Rare haplotypes (represented by <3 copies) were removed, plus sequences much longer or shorter than expected, and then aligned with the remaining haplotypes using clustal W in Bioedit. We then edited the alignment manually to remove indels and match reference sequences.

The sequences were clustered into MOTUs in the program jMOTU (Jones *et al*. 2011) and tested at thresholds from 1-10 bp. A graph of recovered MOTU vs threshold suggests that a 4 bp cut-off was most appropriate in this data set (see Razgour *et al.* 2011). Representative sequences for each MOTU were compared to the reference database in BOLD (www. barcodinglife.org) recording highest sequence similarity. A phylogenetic tree was constructed of representative MOTUs and a series of known reference sequences using maximum parsimony (MP) in MEGA 5 (Tamura *et al*. 2011) using 1000 bootstrap replications.

Ecological Analysis

Ecological analyses were performed in EcoSim V.7 (http://grayentsminger.com/ecosim.htm) and we compared extents of niche overlap using Pianka's (1973) measure of resource sharing (10000 simulated matrices) between shrews and skinks and between males and females in each predator species (equation 3 in Razgour *et al*. 2011). Null models were used to test whether niche overlap was greater than expected by chance. We then re-ran these analyses excluding prey that were only eaten by a single predator. Such occasional prey species are, individually, unlikely to have a significant effect on nutrition and hence on any prey overlap.

Dietary specialization and diversity were estimated using Levins' standardized measure of niche breadth and Shannon's diversity index (equations 1 and 2 in Razgour *et al.* 2011).

Prey groups

Representative sequences from each MOTU were compared to sequences in the BOLD reference database and then included, with known references sequences, in a neighbour-joining reconstruction (Figure 1) in MEGA 5 (Tamura *et al*. 2011). The main prey groups were defined in the cladogram (Figure 1) into Lepidoptera, Dictyoptera, Diptera, Araneae and Gastropoda based on both similarity to known references (category 3 classification, Clare *et al*. in review) and clustering with known references sequences in the cladogram. Individual MOTUs which we found in more than 10% of either shrews or skinks were also analyzed separately. The effects of predator species (shrew or skink), length, mass, age class (juvenile or adult), sex, and whether gravid, on consumption of prey groups, were explored within a Generalised Linear Model (GLM) (data in Table S4). Length was treated as a covariate and all other predictors as factors. The second order interaction predator:sex was included. A binomial error distribution was used with a logit link function. All analyses were conducted in the R statistical package version 2.9.2.

Species-specific shrew and skink primers

As the primers used for 454 sequencing did not, in practice, co-amplify either the shrew or skink DNA, species-specific primers were needed in order to determine whether there was intraguild predation between the two predators.

Cytochrome b sequences for the skinks (AF280133) and shrews (JF784171), along with sequences for a broad range of vertebrates know to occur on the island (or their close relatives), were acquired from GenBank and aligned in BioEdit in order to design species-specific primers. NetPrimer (Biosoft International) was used to test primer sequences for potential primer-dimer and hairpins which would reduce primer efficiency. LtF1 (5’-CCG TCC CCT ACA TTG GCA CTG-3’) and LtR1 (5’-ACA GGA GGT GAA GGA GAG ATA CC-3’) were designed to amplify a 140 bp fragment of the skink while SmF1 (5’- TCG GAA TCT GCT TAA TTG CG-3’) and SmR1 (5’- AAT AAC GAA TGA GTC AGC CAT AAT T-3’) were designed to amplify a 134 bp fragment of the shrew. Gradient PCRs were initially run to determine an optimal annealing temperature for amplification of each target species.

Primers were tested for cross-amplification against DNA extracted from both shrews and skinks, from a range of invertebrate taxa collected on Ile aux Aigrettes and identified to order (n=14) and additionally from invertebrates (n=13) and vertebrates (n=10) collected in the UK (see Supplementary Table S2).

Using the Multiplex PCR Kit (Qiagen) PCR conditions were: 1X Master Mix, 0.5 µM each primer , 10% Q solution and 5ng / µL of DNA with an initial denaturation at 95°C for 15 min, 40 cycles of 94 °C for 30 s, 64.5 °C (for LtF/R) and 64 °C (for SmF/R) for 45 s and 72 °C for 30 s, and a final extension at 72 °C for 10 min. DNA samples were each tested twice, with water negatives included. Neither primer pair cross-amplified with any other taxa. Forty eight skink faecal DNA samples were subsequently screened with LtF/R primers and 49 shrew gut content DNA samples were screened with SmF/R primers, using the conditions described above.

Results

Sequence Analysis

Prey DNA was successfully amplified from 42 shrews and 29 skinks, from which 237,402 sequences were recovered. After removal of rare haplotypes we also removed those that were <100bp and >220bp and, using the MID codes, the labelled sequences were assigned to individuals (female shrews n=14, male shrews n=27, one shrew gender unknown, female skinks n=19, male skinks n=10) and aligned using ClustalW in BioEdit. We edited this alignment to a reference sequence to remove indels. This combined screening of data yielded 3001 haplotypes. The primer, MID and adapter sequences were removed for further analysis.

The resulting Fasta files in jMOTU (Jones *et al.* 2011) were analysed following the same procedures employed by Razgour *et al.* (2011) resulting in the recovery of 119 MOTUs, using the 4bp threshold for assignment.

Ecological analyses.

Of the 119 recovered MOTUs, 53 were found in the diet of skinks and 76 from the diet of shrews with 14 shared between the two predators. Within the 53 MOTUs recovered for skinks, 44 were consumed by females, 17 by males and 8 were shared (one could not be assigned to an individual as sequencing did not recover the full MID). Within the 76 MOTUs recovered for shrews, 34 were consumed by females, 52 by males and 10 were shared.

Niche overlap was significantly greater than expected by chance between predator species (Pianka's measure O_{jk}=0.55, p=0.012), between shrew males and females (O_{jk}=0.58, p=0.009) and between skink males and females (O_{jk}=0.70, p<0.001) (but see Discussion). We then reanalysed the data, excluding 95 MOTUs that were only recorded from the diets of one animal (rare prey), leaving 24 MOTUs (out of 119 or 20%) that were consumed at least twice. When prey species detected in only one shrew or skink were excluded (Table S3), prey overlap was shown to be very strong (shrews vs. skinks O_{jk}=0.80, p=0.002, male vs female shrews O_{jk}=0.80, p=0.003, male vs female skinks O_{jk}=0.91, p < 0.0001). Overall, the niche breadth of both predator species was narrow (Levins' measure B_A=0.18 for skinks and B_A =0.20 for shrews) but high in diversity (H=3.54 for skinks and H=3.74 for shrews). Niche breadth and diversity were similar in shrew females (B_A=0.26, H=3.27) and males (B_A=0.30, H=3.53). Niche breadth was larger and higher in diversity in skink females (B_A=0.30, H=3.46) than in skink males (B_A=0.16, H=2.69).

We could not reliably match any sequences to those in BOLD (www.Barcodinglife.org). A phylogenetic reconstruction of representative sequences for each MOTU was seeded with reference sequences (Figure 1) in order to give an indication of taxonomic groups. This showed a large portion of MOTUs clustering phylogenetically with the reference sequences, suggesting genetic relationships. Of these, 36 MOTUs were most similar to lepidopteran sequences in BOLD and were phylogenetically placed in a clade with known lepidopteran sequences. Similarly, 34 MOTUs showed high sequence similarity to representative Dictyoptera in BOLD (termites, cockroaches and mantids), clustered with known Blattaria in the reconstruction, though a few also showed sequence similarity to reference dipteran sequences.

Analysis of consumption of prey groups

The following analyses were on the putative prey groups as defined in Figure 1. Consumption of Diptera was significantly greater in skinks than in shrews ($\chi^2 = 11.9$, df = 1, $P < 0.001$) (Figure 2a), with 41% of skinks found to have consumed Diptera and only 7% of shrews. There was no significant difference in consumption of Gastropoda between shrews and skinks, but male shrews were significantly more likely to consume them than females ($\chi^2 = 4.3$, df = 1, $P = 0.038$) (Figure 2b) with 44% of males having consumed them and only 14% of females. Consumption of Dictyoptera by shrews appeared higher than that of skinks but this was not quite significant ($\chi^2 = 3.3$, df = 1, $P = 0.068$) (Figure 2c) with 63% of shrews having consumed them and 41% of skinks. Consumption of individual MOTUs, numbers 8, 12 and 13 (all in the Dictyoptera group), were consumed by 20%, 24% and 22% of shrews respectively, but not by any skinks. Conversely, consumption of MOTU number 10 (a dipteran) was found to be significantly higher in skinks than in shrews (χ^2=10.1, df=1, P=0.001), with 38% of skinks having consumed them compared to 7% of shrews. Length, age class, mass and whether gravid had no significant effect on consumption of different prey groups.

Species-specific primers

No evidence was found for intraguild predation between the shrews and skinks; none of the shrew gut samples contained skink DNA and none of the skink faecal samples contained shrew DNA.

Discussion

Overall our results demonstrate that prey overlap between the shrews and skinks is strong, particularly so when rare prey, consumed only once (80% of prey species detected), were excluded from the analysis. Both analyses may have been affected by sample size (42 shrews and 29 skinks) but the effects are difficult to predict. Larger samples size would increase the probability that less frequently eaten prey will be shared between predator species, but could also increase the number of new rare MOTU's consumed. Rare species (weak links) in food webs may have little influence individually but collectively can increase stability, and this pattern, of many weak links but a few strong links, is commonly found in generalist predator food webs (e.g. McCann *et al.* 1998). All measures of dietary overlap have been criticised (e.g. Wallace 1981) but when the levels of overlap are so strong they are likely to accurately reflect what is happening in the field. We do not know, however, the degree to which the overlap is driven by prey availability or whether at different times of year prey choices by shrews and skinks change. The fact that so many prey were detected only once implies that both shrews and skinks are adaptable and opportunistic, although more prey species may be shown to be shared by the two predators with more sampling. Similarly, species-level analyses of the diets of bats in previous studies showed rare species comprising approx. 50-90% of recovered MOTUs (Clare *et al*. 2009, 2011; Bohmann *et al.* 2011). Strong niche overlap does not necessarily imply significant competition if prey are numerous and not limiting. However, Seymour *et al.* (2005) provided indirect evidence that prey availability can be limiting, by showing that the mean biomass of shrews increased when their numbers were reduced. It is possible, however, that shrew biomass increased for other reasons, such as reduced intensity of social interactions or changes in abiotic conditions. Our field study coincided with when invertebrate resources are considered to be relatively abundant in comparison to other times of year (Cole & Harris 2011).

Although none of the prey could be conclusively identified to a specific taxon, the MOTU approach provided an elegant means of testing for niche overlap between the two predators and between sexes of each predator species, even without access to a reference collection. Data on precisely which prey species are being exploited, particularly those consumed by both shrews and skinks, would require a major barcoding exercise of taxa within the groups indicated on the tree (Figure 1). This would need to be combined with a major effort by museum taxonomists to identify all the taxa morphologically to species. This would not be difficult in, for example, Europe or North America, where the fauna are less diverse and well-studied, but in tropical systems it would present a significant challenge. Only if this were done could the MOTUs found amongst the diets of the shrews and skinks be retrospectively assigned to species. However, analysis of our putative assignments defined in Figure 1 did show some interesting differences. Although Lepidoptera were eaten by both predators, skinks were approximately six times as likely to have consumed Diptera as shrews (Figure 2a). The near significantly greater consumption of Dictyoptera by shrews may relate to Blattaria (Figure 2c), although these have been reported to be eaten by both skinks (Vinson & Vinson 1969; Pernetta *et al.* 2005; Richards 2007; Zuël 2009; Copsey *et al*. 2011) and shrews (Advani & Rana 1981). Dictyoptera are a superorder containing a large range of ecologically very different taxa (termites, cockroaches and mantids), thus possibly masking dietary differences at the group level.

Shrews and skinks clearly have very different physiologies and it might be predicted that the homeothermic shrews would digest their prey more rapidly than poikilotheric skinks. However, we were able to access the shrew samples from an earlier stage of digestion (the stomach) while the skink diet was analysed from fresh faeces. What combined effects these may have had on prey detection, and the relative abundance of different MOTU consumed, could only be established through captive feeding trials.

Some differences were found between sexes, for example female skinks ate a greater diversity of prey species than males, but the reasons for this, though intriguing, are not known. It may be that the dietary needs of reproducing females are different to those of males. Sex differences in diet are often related to sexual dimorphism, for example in birds and mammals (e.g. Rosalino *et al*. 2009; Phillips *et al.* 2011) and arthropods (e.g. Symondson & Liddell 1993; Pekár *et al*. 2011), where the size difference allows predators to access different prey, allowing intersexual partitioning of resources. Adult male skinks and shrews are larger than females. Male shrews were more than three times as likely to have eaten gastropods than females (Figure 2b). However, all of these results would have been affected by the differences in sample sizes and they would require further work to verify.

Analysis with species-specific primers provided no evidence of direct intraguild predation by shrews on skinks or skinks on shrews. However, this contrasts with observations on the island of juvenile skink remains in the guts of shrews and shrew remains in the faeces of skinks (pelts and hair), plus direct observations of mutual predation (N. Cole and D. Vencatasamy pers. obs.). Unavoidable delays in conducting our work meant that shrews and skinks were sampled well after the peak period when skinks hatch and are at their most vulnerable. The release of Telfair's skinks onto Ile aux Aigrettes coincided with substantial declines in the abundance of shrews, possibly as a result of skinks preying on shrews. However, at the current low shrew density dietary evidence of predation may not be detected unless the number of skinks sampled was greatly increased. If prey are limiting then high prey overlap between shrews and skinks may also have played a role in the decline of the shrews.

Any form of analysis of predation, whether morphological or utilising PCR, must always be qualified by the fact that we cannot distinguish between predation, scavenging and secondary predation. Scavenging of dead material by insect predators has been shown to be a likely source of error using PCR (Foltan *et al*. 2005; Juen & Traugott 2005). Within invertebrate food webs, secondary predation, where one predator eats another and the prey in the guts of the consumed predator can be detected, is probably a less important source of error (Sheppard *et al.* 2005). In all cases (predation, scavenging, secondary predation) the prey detected are contributing to the nutrition of the predator but the dynamics of the interactions are clearly very different.

The novel combination of existing primers proved to be highly effective at amplifying invertebrate DNA, covering a broad range of invertebrates but with no co-amplification of the predators. They proved to be a significant improvement on the Uni-MiniBar primers of Meusnier *et al.* (2008), UniMinibarF1 / UniMinibarR1, which have been criticised for their low taxonomic coverage (Ficetola *et al.* 2010). However, when is UniMinibarR1 combined with the general invertebrate forward primer LCO-1490 of Folmer *et al.* (1994) specificity and coverage were excellent.

As far as we are aware, this is only the second time that PCR has been used to analyse reptile diets from faecal samples, the first being our previous study of predation on earthworms by slow worms, the legless lizards *Anguis fragilis* (Brown *et al.* 2008). In that instance the primers used for NGS were the earthworm group-specific primers developed by Harper *et al.* (2005). A further paper on the diets of snakes in this special issue reports the vertebrate and invertebrate diet of the smooth snake *Coronella austriaca*, analysed using prey-specific primers (Brown *et al*. submitted). The fact that PCR and NGS can be used to analyse the diets of reptiles from faeces, despite the fact that many species digest their prey to the extent of dissolving bones (Secor 2008), opens up a potentially rich field for future research on reptile trophic ecology. A different molecular approach was taken recently by Goiran *et al.* (2013), who demonstrated that fish eggs palpated from the stomachs of sea snakes could be identified by sequencing their DNA.

Concerted trapping in 1999 to eradicate the shrews from Ile aux Aigrettes was only partially effective. Some individuals are 'trap-shy' and can go on to generate a resurgent population within a short time. It appears to be the case that shrews enter traps through curiosity, rather than responding to baits (which are often left untouched) (Varnham *et al*. 2002; Seymour *et al.* 2005). Thus analysis of their diets in the field provided an opportunity to identify favoured prey that, as bait or food odours, could improve trap efficiency. The results of our analysis suggest that Lepidoptera larvae or cockroaches may provide effective bait. Cockroach frass from laboratory cultures is highly pungent and may be sufficient to attract shrews.

The ethics of killing vertebrates in order to obtain gut samples must be properly justified. Here we caught and killed shrews in the field (using UK Home Office approved techniques), to obtain gut samples. Once caught it was not considered ethically acceptable to release these pests back to the wild, where they would continue to pose a threat to native wildlife. This allowed us to maximise the information obtainable from these animals by analysing their stomach contents (rather than faeces) where prey DNA was likely to be less degraded. A key aim of Mauritian conservationists has been to eradicate shrews from offshore islands to permit further restoration processes. However, to date, eradication attempts have only been successful using traps on topographically simple islands of a few hectares or less (Varnham *et al*. 2002). The problem with the shrews is that traps do not catch them efficiently and suitable poison baits have not been devised (Varnham *et al*. 2002; Seymour *et al*. 2005).

Our conclusion, therefore, is that shrews and skinks are feeding to a large extent on the same species of invertebrate prey, potentially leading to competition. If so then shrew control is likely to be beneficial to the fitness of the skinks. Mutual predation is known to occur, but our analysis failed to find evidence of this outside the period when juvenile skinks are particularly vulnerable. This is probably because skinks grow too large to be attacked by shrews and similarly, at low densities, shrews increase in biomass (Seymour *et al.* 2005) and may be too large for predation by skinks. Given that the shrews pose a threat to island biodiversity, development of methods to eradicate them from islands such as Ile aux Aigrettes should continue to be a priority.

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Data accessibility

All sequences arising from NGS, fully processed, collapsed and aligned, plus allocated to individual predators and ready for analysis, will be included as Online Supplementary Material after acceptance of the paper. Three files will be included: all ‘sequences pooled.fasta’, ‘all sequences shrew.fas’ and ‘all sequences skink.fas’.

Author Contributions Box

Gut and faecal samples were collected from Mauritius by RB and DV, and DNA extracted by RB, who designed and applied species-specific primers for analysing mutual predation between shrews and skinks. Preparation of samples for NGS was conducted by DSB, along with analyses of predation on key prey taxa. Bioinformatics and ecological analyses were conducted by ELC. Supervision of the fieldwork in Mauritius was conducted by NC, who provided the expertise on Mauritian ecology. AM conducted the 454 analysis. Overall supervision of the project and the writing of the paper were primarily conducted by WOCS, with major contributions from co-authors.

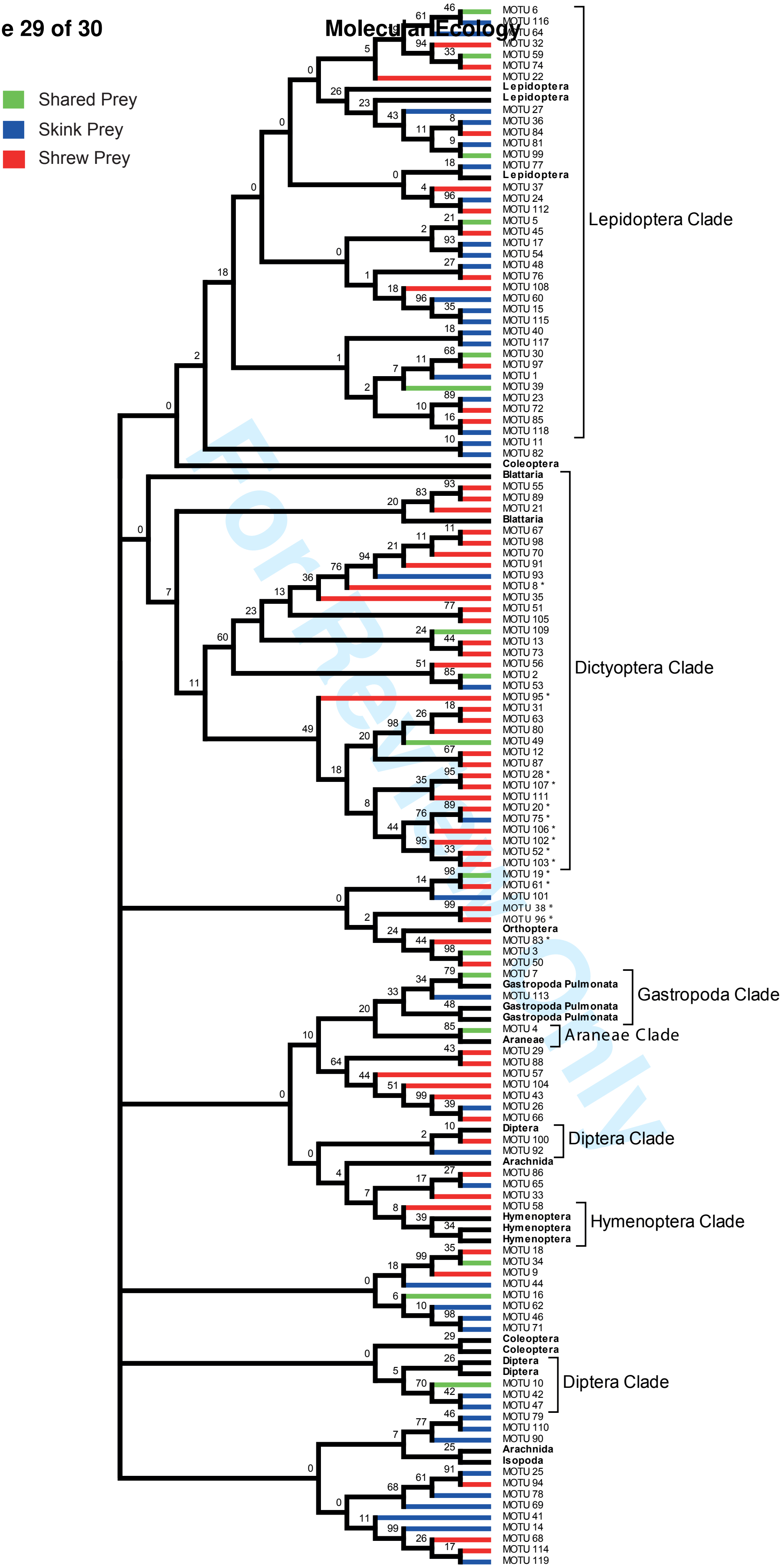
Figure legends

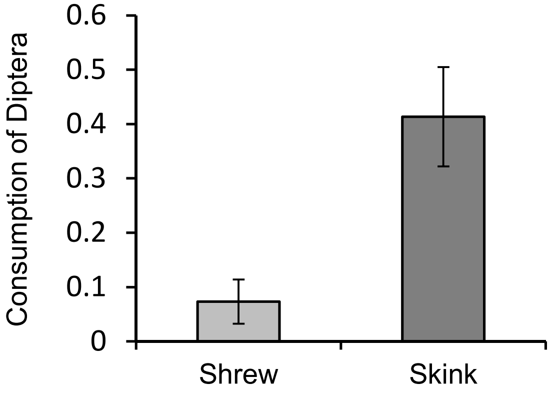
Figure 1

Cladogram showing reconstructed relationships between all MOTUs retrieved from the guts or faecal samples of Asian Musk Shrew and Telfair's Skinks, colour codes to denote prey consumed by shrews, skinks or by both species.

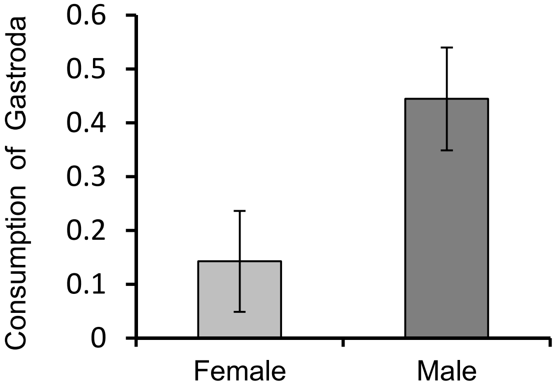
Figure 2

Main significant or near significant differences in diet arising from analysis of putative higher-order classifications, as defined in Figure 1. **a.** Predicted probability of consumption of Diptera (± s.e.) by shrews and skinks, showing significantly higher consumption in skinks ($p < 0.001$). **b.** Predicted probability of consumption of Gastropoda (± s.e.) by shrews, showing significantly higher consumption in males than in females ($p = 0.038$). **c.** Predicted probability of consumption of Dictyoptera (± s.e.) by shrews and skinks, showing a trend towards higher consumption by shrews ($p = 0.068$).

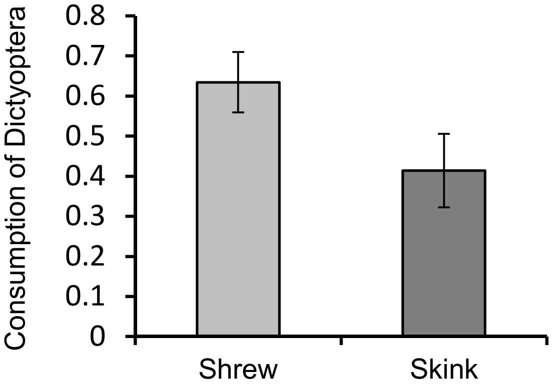

Shared Prey
Skink Prey
Shrew Prey
MOTU 6
MOTU 116
MOTU 64
MOTU 32
MOTU 59
MOTU 74
MOTU 22
Lepidoptera
Lepidoptera
MOTU 27
MOTU 36
MOTU 84
MOTU 81
MOTU 99
MOTU 77
Lepidoptera
MOTU 37
MOTU 24
MOTU 112
MOTU 5
MOTU 45
MOTU 17
MOTU 54
MOTU 48
MOTU 76
MOTU 108
MOTU 60
MOTU 15
MOTU 115
MOTU 40
MOTU 117
MOTU 30
MOTU 97
MOTU 1
MOTU 39
MOTU 23
MOTU 72
MOTU 85
MOTU 118
MOTU 11
MOTU 82
Lepidoptera Clade
Coleoptera
Blattaria
MOTU 55
MOTU 89
MOTU 21
Blattaria
MOTU 67
MOTU 98
MOTU 70
MOTU 91
MOTU 93
MOTU 8 *
MOTU 35
MOTU 51
MOTU 105
MOTU 109
MOTU 13
MOTU 73
MOTU 56
MOTU 2
MOTU 53
MOTU 95 *
MOTU 31
MOTU 63
MOTU 80
MOTU 49
MOTU 12
MOTU 87
MOTU 28 *
MOTU 107 *
MOTU 111
MOTU 20 *
MOTU 75 *
MOTU 106 *
MOTU 102 *
MOTU 52 *
MOTU 103 *
Dictyoptera Clade
MOTU 19 *
MOTU 61 *
MOTU 101
MOTU 38 *
MOTU 96 *
Orthoptera
MOTU 83 *
MOTU 3
MOTU 50
MOTU 7
Gastropoda Pulmonata
MOTU 113
Gastropoda Pulmonata
Gastropoda Pulmonata
Gastropoda Clade
MOTU 4
Araneae
Araneae Clade
MOTU 29
MOTU 88
MOTU 57
MOTU 104
MOTU 43
MOTU 26
MOTU 66
Diptera
MOTU 100
MOTU 92
Diptera Clade
Arachnida
MOTU 86
MOTU 65
MOTU 33
MOTU 58
Hymenoptera
Hymenoptera
Hymenoptera
Hymenoptera Clade
MOTU 18
MOTU 34
MOTU 9
MOTU 44
MOTU 16
MOTU 62
MOTU 46
MOTU 71
Coleoptera
Coleoptera
Diptera
Diptera
MOTU 10
MOTU 42
MOTU 47
Diptera Clade
MOTU 79
MOTU 110
MOTU 90
Arachnida
Isopoda
MOTU 25
MOTU 94
MOTU 78
MOTU 69
MOTU 41
MOTU 14
MOTU 68
MOTU 114
MOTU 119


Consumption of Diptera
0.6
0.5
0.4
0.3
0.2
0.1
0
Shrew
Skink

2a


Consumption of Gastroda
0.6
0.5
0.4
0.3
0.2
0.1
0
Female
Male

2b


Consumption of Dictyoptera
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Shrew
Skink

2c

SUPPLEMENTARY MATERIAL

Table S1

Invertebrates collected from Ile aux Aigrettes and tested for PCR amplification with the two primers sets developed for 454 pyrosequencing, LCO-1490 / Uni-MiniBar-R and LCO-1490 / ZBJ-ArtR2c.

Potential prey	**LCO-1490 / Uni-MiniBar-R**	**LCO-1490 / ZBJ-ArtR2c**
Coleoptera 1	**√**	**√**
Oligochaeta	**√**	**√**
Hemiptera 1	**√**	**√**
Isopoda	**√**	**√**
Dermaptera	**√**	**√**
Embioptera	**√**	**√**
Diplopoda	**√**	**√**
Hymenoptera (*Vespa sp.*)	**√**	**√**
Araneae 1	**√**	**√**
Gastropoda 1	**√**	**√**
Lepidoptera 1	**√**	**√**
Diptera	**√**	**√**
Blattaria 1	**√**	**√**
Odonata	**√**	**√**
Decapoda	**√**	**√**
Gastropoda 2	**√**	**√**

Hymenoptera - Formicoidea	**√**	**√**
Lepidoptera 2	**√**	**√**
Scorpiones	**√**	**√**
Araneae 2	**√**	**√**
Coleoptera - Cerambycidae	**√**	**√**
Diptera - Culicidae	**√**	**√**
Collembola	**√**	**√**
Orthoptera - Gryllidae		**√**
Hemiptera 2	**√**	**√**
Hemiptera 3	**√**	
Chilopoda	**√**	**√**
Coleoptera 2	**√**	
Blattaria 2	**√**	**√**
Total	**28/29**	**27/29**

Table S2.

Non-target species tested for cross-amplification with skink-specific (LtF/R) and shrew-specific (SmF/R) PCR primers. Neither primer set co-amplified any of these taxa.

Order	**Species**	**Origin**
Coleoptera	spp. x2	Ile aux Aigrettes
Lepidoptera	spp. x2	Ile aux Aigrettes
Blattaria	spp. x2	Ile aux Aigrettes
Hymenoptera	spp. x2	Ile aux Aigrettes
Diptera	spp. x2	Ile aux Aigrettes
Isopoda	spp. x2	Ile aux Aigrettes
Aranaea	spp. x2	Ile aux Aigrettes
Pulmonata	*Arion intermedius*	UK
	A. distinctus	UK
	Limax flavus	UK
Haplotaxida	*Lumbricus terrestris*	UK
	L. rubellus	UK
	Aporrectodea caliginosa	UK
	A. longa	UK
Coleoptera	*Notiophilus biguttaus*	UK
	Adalia bipunctata	UK
	Tachyporus obtusus	UK
Diptera	*Tipulidae sp.*	UK

Dermaptera	*Forficula sp.*	UK
Aranaea	*Erigone ddentipalpis*	UK
Squamata	*Zootoca vivipara*	UK
	Anguis fragilis	UK
	Coronella austriaca	UK
	Natrix natrix	UK
Rodentia	*Myodes glareolus*	UK
	Mus musculus	UK
	Apodemus flavicollis	UK
Soricomorpha	*Neomys fodiens*	UK
	Sorex araneus	UK
Caudata	*Lissotriton helveticus*	UK

Table S3

Numbers of shrews and skinks, of each sex, that contained each prey MOTU, excluding MOTUs that were only found in one animal overall. Shrew N/R is an animal not sexed (see text). ‘Total detections’ are the numbers of shrews+skinks testing positive for that MOTU. For a complete list, and to find MOTU numbers, see Figure 1.

MOTU no.	Skinks male	Skinks female	Shrews male	Shrew female	Shrew N/R	Total detections
2	3	7	10	6	1	27
3	1	1	0	2	0	4
4	3	3	6	5	1	18
5	1	6	10	5	0	22
6	3	5	5	0	0	13
7	1	5	10	3	1	20
8	0	0	3	5	1	9
10	3	8	3	0	0	14
11	0	0	3	0	0	3
12	0	0	7	3	1	11
13	0	0	6	3	1	10
16	0	0	2	0	0	2
20	0	0	3	0	0	3
21	0	0	4	1	0	5

28	0	0	3	0	0	3
30	0	0	1	1	0	2
31	0	0	3	0	0	3
34	0	1	3	2	0	6
39	1	2	2	2	0	7
44	1	3	0	1	0	5
49	0	1	0	1	0	2
59	0	1	1	0	0	2
71	1	1	0	0	0	2
116	0	2	0	0	0	2

Table S4

File ‘MOTUs consumed by shrews and skinks.xls’. Spreadsheet providing raw data on the shrews and skinks from which we successfully amplified invertebrate DNA, including sex, mass, length, adult/juvenile status and whether gravid.

Tables S5-S6

Spreadsheets including representative sequences for all haplotypes arising from NGS, fully processed, collapsed and aligned, allocated to individual predators and ready for analysis. Divided into ‘All sequences shrew.fas’ and ‘All sequences skink.fas’.

	Skink		**Shrew**		
MOTU	**Males**	**Females**	**Males**	**Females**	**Unknown**
2	11, 31, 46	20, 33, 36, 41, 45, 7, 9	11, 13, 21, 26, 33, 36, 44, 6, 8, 9	1, 25, 28, 29, 7, 37	2
3	42	20		7, 32	
4	10, 31, 42	3, 7, 9	12, 22, 26, 40, 41, 44	19, 20, 38, 39, 32	2
5	11	20, 29, 2, 36, 41, 44	13, 15, 21, 26, 33, 35, 44, 6, 8, 9	25, 29, 38, 7, 50	
6	11, 15, 42	12, 18, 20, 36, 41	12, 21, 22, 26, 33		
7	39	18, 20, 33, 41, 48	14, 17, 22, 30, 34, 3, 41, 43, 44, 9	45, 46, 49	2
8			21, 28, 8	1, 25, 7, 32, 37	2
10	11, 43, 6	14, 2, 33, 3, 40, 41, 44, 45	15, 40, 44		
11			41, 44, 9		
12			15, 16, 17, 26, 33, 41, 48	24, 25, 49	2
13			21, 33, 36, 44, 8, 9	25, 28, 37	2
16			40, 41		
20			26, 33, 48		
21			22, 26, 48, 46	49	
28			26, 33, 48		
30			41	29	
31			26, 33, 48		
34		9	40, 44, 9	37, 50	
39	42	4, 9	40, 44	4, 33	
44	37	33, 41, 9		49	
49		18		24	
59		7	6		
71	11	41			
116		12, 41			