Directional dispersal has not evolved during the cane toad invasion

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Summary

1. The ability to disperse along a consistent compass heading strongly affects the rate and efficiency of an animal’s displacement, and thus is under selection at the expanding edge of a biological invasion.

2. We used radio-telemetry to assess whether the dispersal direction of cane toads (*Rhinella marina*) changed as a function of time since invasion, by comparing (1) toads at a single site, monitored annually for 10 years subsequent to toad arrival; (2) toads collected from sites across the species’ invaded range in Australia, and radio-tracked at a common site; and (3) the offspring of those transported toads, that were reared in captivity under common-garden conditions.

3. The first of these data sets showed non-random directionality, indicating strong spatial sorting operating on this trait: toads moved in a northwesterly direction for the first four years post-invasion, but in random directions thereafter. Despite the evidence for trait sorting, no consistent directionality was seen in toads relocated from populations with different invasion histories, nor in their offspring. Why do we see no evolutionary shifts?

4. Dispersal directionality of the offspring was not correlated with that of their parents, arguing against a genetic basis to this behavioural trait. Thus, while an expanding invasion front creates an evolutionary pressure for animals to move in a specific direction, evolution of this trait has not occurred in this system because directionality is not heritable.

5. The observed northwesterly movements of toads at the invasion front were due to simple density differentials: in the first few years, most toads arriving at our study site originated from earlier-colonised (and hence denser) populations to the southeast.

Key-words: *Bufo marinus*, invasive species, range expansion, spatial sorting, orientation, dispersal.
Introduction

The evolutionary consequences of processes such as climate change, biological invasion and landscape fragmentation on species' mobility (e.g. migration, dispersal) are attracting increased scientific attention (Thomas et al. 2001; Travis & Dytham 2002; Root et al. 2003; Fahrig 2007; Gienapp et al. 2008; Visser 2008). An organism's rate of displacement is influenced by numerous traits, such as speed, stamina, frequency of movement, and path straightness (Fahrig 2007; Bartoń et al. 2009; Ducatez et al. 2012). While there is mounting evidence that these traits are often heritable (e.g. Phillips, Brown & Shine 2010a; Forsman, Merilä & Ebenhard 2011; Larranaga et al. 2013; Lombaert et al. 2014), understanding the extent to which such traits are inherited, as opposed to plastic, is a prerequisite to predicting their evolutionary responses.

Individuals at the expanding edge of an invasion front are subject to novel evolutionary forces, which often result in an evolved increase in dispersal rates (Phillips, Brown & Shine 2010b; Perkins et al. 2013). When dispersal traits are heritable, genes that code for more rapid dispersal accumulate at the invasion front either through natural selection (reflecting a fitness advantage to individuals in the vanguard: Brown, Kelehear & Shine 2013) or spatial sorting (reflecting a trend for faster individuals to dominate the front, simply because of their greater speed: Shine, Brown & Phillips 2011). Where the traits are not heritable we may still see spatial sorting and natural selection occurring, but these will not be accompanied by resultant trait evolution.

Over any spatial scale, the ability to move in a straight path is perhaps the most obvious way to economise movement between two points (Doerr & Doerr 2005; Fahrig 2007; Bartoń et al. 2009). Straight-line movement are enhanced either by a propensity for autocorrelated headings (“keep moving in the same direction as you did yesterday”) or a preference for a
specific compass direction (“keep moving westwards”: Bartoń et al. 2009). Both of these mechanisms are plausible; heritability and response to selection have been demonstrated for correlated headings (in toads: Brown, Phillips & Shine 2014) and for compass orientation (in migrating birds: Helbig et al. 1994). In practical terms, it is difficult to tease these two factors apart, but biological invasions may provide an opportunity to do so. To test whether preferred dispersal direction responds to selection, we need an invasion front that has been expanding in a consistent direction for many generations. If dispersal direction is heritable we would expect individuals that move in the same direction as the invasion front to rapidly dominate the vanguard population. This is not to imply that selection should favour directionality for dispersal in other contexts (e.g. dispersal to reduce inbreeding or kin competition), only that if displacement direction has a genetic component, the trait should accumulate at the expanding edge of a directional invasion.

Our study system for examining this question involves the continuing expansion of feral cane toads (Rhinella marina Linnaeus 1758) across tropical Australia. Since their introduction to northeastern Australia in 1935, the toads have spread westwards at an increasing rate (from <15 km/yr to >50 km/yr: Phillips et al. 2006). These strong shifts in dispersal rate are caused by changes to several aspects of toad behaviour (Phillips et al. 2008; Alford et al. 2009; Lindström et al. 2013). Research on common-garden-raised progeny of toads from different sites has demonstrated significant heritability of overall dispersal rate (Phillips et al. 2010a), and specifically of path straightness (Brown et al. 2014). We gathered three datasets to assess whether compass headings of cane toad movement vary as a function of invasion history: (1) from annual radio-tracking of toads at a single site, for 10 years following the initial arrival of the invasive anurans; (2) from toads collected at a range of sites across the species’ Australian range (from invasion-front to long-colonised), and translocated to a common site for radio-tracking; and (3) from the progeny of the translocated toads, reared under standardised
conditions to reduce spatially-variable environmental influences on dispersal behaviour. We measured the compass headings of each movement of dispersing toads to ask:

1. Do toads at the forefront of the invasion tend to move in a specific direction?
2. When tracked in the same location, do toads from more western (frontal) locations show a tendency for westerly dispersal relative to toads from eastern (long-established) locations?
3. Is the dispersal direction of the offspring of those toads influenced by the locality where their parents were collected?
4. Do offspring show a similar dispersal direction to their parents?

**Materials and methods**

**STUDY SPECIES**

Native to southern and central America, cane toads were translocated from the Caribbean to Hawaii, and thence to Australia, in a misguided attempt to control insect pests in commercial sugarcane plantations (Lever 2001). Progeny from 101 toads were released along the northeastern coast of Queensland in 1935, and have since spread widely. The annual rate of range expansion has accelerated about five-fold (Phillips et al. 2006), reflecting more frequent and longer-distance movements by invasion-vanguard animals (see above).

**STUDY SITE AND FIELD METHODS**
Data for the present study were gathered at Middle Point on the Adelaide River floodplain, 60 km east of the city of Darwin, in the wet-dry tropics of northern Australia (12.6°S, 131.3°E). Temperatures are high all year, but rainfall is concentrated in a four-month “wet-season” (December to March). Cane toads arrived at this site in 2005 (Brown et al. 2006). Adult toads are nocturnal and although they remain active year-round, they disperse only during the wet-season (Brown, Kelehear & Shine 2011). All toads in this study were radio-tracked at a common site, on a pastoral property (Beatrice Hill Farm) 5 km from our field station. Toads were fitted with a bead-chain waist-belt holding a small (<5% of toad body mass) radio-transmitter (model PD2, Holohil Systems, Ottawa, Canada). Toads were then radio-tracked each day, usually for a period of 5 days, and the coordinates of their daily locations were read with a handheld GPS (Etrex 10; Garmin, Kansas City, Missouri). The radio-tracking data presented here come from three groups of toads (detailed below): wild toads captured in the study site, wild toads captured at different sites and translocated to our study site for radio tracking, and captive-reared offspring from these translocated toads.

WILD LOCAL TOADS

Every year since toads first arrived at Beatrice Hill Farm (2005), we have radio-tracked wild-caught adults. These toads were captured during night-time surveys and returned to the lab for measuring and marking. They were equipped with radio-transmitters and released at their point of capture the following evening. Most toads were tracked for a pre-defined period of 5 days, but others were tracked for longer, variable periods. For the present purposes we only used the first 5 days of location data per individual, and we excluded any individuals tracked for periods of less than 5 days. During 10 years of mark-recapture on >5000 individuals, we
have never recorded a toad being present in the study site for more than a single year. Thus, the samples of toads tracked over different periods are independent, in that no individual toad was ever tracked during more than one period. Ten years post-invasion, toads at our study site are still dispersive rather than resident (Brown & Shine 2014). They remain in the area during the dry-season, around sources of water, but with the onset of wet-season rains they disperse, apparently never to return.

TRANSLOCATED TOADS

During September 2006, adult toads were collected from four sites across a 1636-km transect spanning the cane toads’ invaded range over northern Australia, from Queensland (QLD) to the Northern Territory (NT; Fig. 1). The easternmost site, Cairns QLD, was the location of the toads’ initial release in 1935. The second site, Normanton QLD (505 km west of Cairns), was invaded by toads in 1964. Borroloola NT, the third collection site, is 1013 km west of Cairns and was invaded by toads in 1984. The final collection site, Timber Creek NT, was the frontline of the toad invasion at the time of the collections in 2006, and is 1636 km west of Cairns. The animals were transported to our field station at Middle Point and maintained in captivity until January 2007, at which time each was equipped with a radio-transmitter and released at the same site where we tracked wild toads (see above). The toads were tracked over 5 days and their locations recorded each day. Following the 5 days of radio-tracking, the toads were recaptured and returned to captivity. Subsequently, these adult toads were bred with one another to produce within-population as well as among-population crosses. The progeny of these pairings were raised under common, standardised conditions to reduce environmental effects on phenotypic traits (see Phillips et al. 2010a for details).
In 2009, when the progeny produced from breeding the translocated toads reached adult size, we released and radio-tracked them at the same site where their parents had been tracked 2 years earlier. To quantify the genetic origin of each toad, relative to the invasion history of its parents, we assigned each offspring a 'mid-parent distance', calculated as the average of maternal and paternal collection localities, expressed as distance from the eastern (origin) end of the transect (Cairns, QLD). Thus, an offspring of a Cairns mother and a Cairns father would have a mid-parent distance value of 0 km, while the offspring of a Cairns mother and Timber Creek father would have a mid-parent distance value of 818 km, and an offspring with two Timber Creek parents a value of 1636 km. This mid-parent distance metric is directly analogous to values of "crossing (or pollination or maternal) distance" used in common garden studies, typically for assessing in/out-breeding depression or identifying source populations for restoration ecology projects (Verhoeven et al. 2011; Maschinski et al. 2013).

We only used data from toads that moved at least three times during the 5-day radio-tracking period. This restriction left us with data from 254 field-collected local toads, 82 translocated toads, and 129 common-garden-raised progeny of the translocated toads.
We assessed the compass heading of toad movements divorced from the actual distance moved at that heading. Elsewhere we have used our radio-telemetry data to document dramatic effects of invasion history on the displacement rates of toads (Brown et al. 2013, Phillips et al. 2008, Phillips et al. 2010a). Because invasion front toads move much further and more often than toads from established populations, the greater distances magnify the net directionality of overall displacement. For the present purposes, the focus of our interest is the consistency of headings chosen by toads, not in the distances they moved. Thus we investigate directionality in isolation from distances moved. The variable of interest to us, the compass heading of movements, is measured on a circular scale. Because conventional linear statistical methods thus are inappropriate, we used circular statistical tests in our analyses. The initial step was to calculate a mean direction of movement for each toad based on the headings of three to five moves over the tracking period. These individual means were then averaged to give grand means for each group.

To examine temporal changes in movement among local toads we divided data into five periods, each of 2 years in duration (2006-7, 2007-8, 2009-10, 2011-12, 2013-14). To examine differences in movement direction of toads captured in different locations and brought to Beatrice Hill Farm for radio-tracking, we grouped individuals by their location of origin (Cairns, Normanton, Borroloola, Timber Creek). To assess the effects of parental location on movement of progeny, we divided offspring into four groups based on their mid-parent distances from Cairns: 0 km (Cairns*Cairns crosses = 0 km), 500 km (Normanton*Normanton = 505 km, Borroloola*Cairns = 506 km), 1000 km (Cairns*Timber Creek = 818 km, Borroloola*Borroloola = 1013 km, Normanton*Timber Creek = 1070 km), and >1500 km (Timber Creek*Timber Creek = 1636 km).

In some cases, testing our predictions involved comparing observed mean directions to specific, expected directions. For this purpose we used V-tests (Zar 2010), which are
equivalent to one-sample \( t \)-tests, to detect whether toads moved at the specified heading. Under the prediction that the process of invasion would have selected for westward directionality in toads, the expected angle for the V-test was 270°. Under the prediction that translocated toads would home to their capture locations, the expected headings were: 109° (Cairns), 120° (Normanton), 125° (Borroloola), 194° (Timber Creek). For other predictions we had no \( a \) \textit{priori} expectations of specific headings, but merely wished to assess whether toads showed any significant (i.e. non-random) heading at all. We used Hotelling’s one-sample second-order tests (Zar 2010) for this purpose. To compare whether the mean heading differed between pairs of groups of toads we used Hotelling’s two-sample second order tests (Zar 2010). To assess whether the direction of movement of offspring resembled that of their parents, we calculated angular-angular correlations (Zar 2010). We carried out circular statistical analyses using Oriana 4.0 (Kovach Computing Services, Wales, UK) and the R package ‘circular’ (Agostinelli & Lund 2013).

**Results**

(1) **DOES THE DIRECTION OF DISPERSAL CHANGE OVER THE COURSE OF INVASION AMONG LOCAL TOADS?**

Prior to 2005, the frontline of the toad invasion was located southeast (SE) of our study site and advancing at a heading of approximately 310° (see Fig. 1 in Phillips \textit{et al.} 2007). The first toads that invaded our study site (2005-6) moved with significant directionality to the northwest (NW; mean heading of 346°; Table 1, Fig. 2a). During this initial phase of invasion, no toads were observed to move SE, at any heading between 117° and 177°. During the
second 2-year period of the invasion (2007-8), toads still moved NW with significant
directionality, and at a heading (1°) that was statistically indistinguishable from that recorded
in 2005-6 (Table 1). Nonetheless, variation in headings was greater (Table 1, Fig. 2b); most
toads moved towards the NW, but several moved SE, counter to the direction of movement of
the invasion front. After the fourth year of the invasion, average movement of toads no longer
displayed significant directionality, with toads moving at headings around the compass (Table
1, Fig. 2c-e). Many individual toads still followed straight, directional paths; but for every
toad heading north, there was one moving south, negating each other’s net directionality.

Thus, over the first 10 years post-invasion, toads showed a decreasing tendency to move
in a common direction. Pairwise comparisons of mean dispersal directions among the five
time periods revealed that the grand mean directions of movement did not differ significantly
between the first and second periods (349° and 1° respectively), but both of those values
differed from the grand mean directions during the subsequent three periods (Table 1). The
circular variance around the grand mean heading for each time period increased over the 10-
year study (Fig. 3), reflecting the erosion of a common directionality over time.

(2) DOES DIRECTION OF MOVEMENT VARY AMONG TOADS TRANSLOCATED
FROM DIFFERENT POPULATIONS?

None of the populations of translocated toads showed a significant tendency to head
westwards (270°) after being brought to Middle Point for radio-tracking (V-tests; all \( u < 1.28, \)
all \( P > 0.10 \)). There was also no evidence that any of the translocated groups attempted to
home back to their population of origin (V-tests; all \( u < 1.55, all \( P > 0.06 \)), though toads from
Borroloola showed a marginally significant (\( P = 0.06 \)) tendency; their mean heading was 89°
and their home was at a heading of $125^\circ$. Overall, the four groups of toads moved in random
directions, with no overall heading consistency in any group (Hotelling's one sample tests; all
$F < 2.16$, all $P > 0.15$; Table 2, Fig. 4). Pairwise comparisons of mean heading among the
four groups indicated no significant differences among them (i.e. all were non-directional,
Hotelling's two-sample tests; all $F < 2.13$, all $P > 0.15$; Table 2).

(3) DOES DIRECTION OF MOVEMENT DIFFER AMONG OFFSPRING FROM
DIFFERENT POPULATIONS?

Only the progeny of toads originating from Cairns (mid-parent distance group = 0 km), which
moved at a mean heading of $268^\circ$, showed a significant predilection for westerly movement
(V-test; $u = 1.69$, $P = 0.046$; Fig. 5a). Toads in the other three mid-parent distance groups did
not exhibit overtly westerly movement (V-tests; all $u < 1.03$, all $P > 0.15$). The more general
test for non-random directionality (as opposed to tests for deviance from a specified heading),
revealed no overt directionality in any of the four mid-parent distance groups (Hotelling's
one-sample tests; all $F < 2.26$, all $P > 0.13$; Table 3, Fig. 5). Pairwise tests indicated a
significant difference in mean direction between 0 km and 500 km distance groups ($267.7^\circ$ vs.
$11.2^\circ$; Hotelling's $F = 4.62$, $P = 0.024$), but no significant differences in mean headings
among pairs of other mid-parent distance groups (Hotelling's two-sample tests; all $F < 0.57$,
all $P > 0.57$; Table 3).

(4) DO OFFSPRING MOVE IN THE SAME DIRECTION AS THEIR PARENTS?
The directions travelled by the 129 offspring were not correlated with the directions travelled by their mothers ($r = 0.13, P = 0.13$), or by their fathers ($r = 0.17, P = 0.07$), and nor were they correlated with the average of the parental directions ($r = 0.13, P = 0.19$).

Discussion

Elsewhere, we have shown that the path straightness of dispersing toads varies as a function of invasion history (frontal toads move in straighter lines), and is similar between parents and their offspring (Brown et al. 2014). The predilection of frontal toads to consistently move in straight lines argues that they possess adequate sensory and cognitive apparati to repeatedly orient themselves along a compass heading. Indeed, the ability of toads to navigate and orient has been well studied (e.g. Adler & Taylor 1981; Sinsch 1988; Semlitsch 2008; Homan et al. 2010; Santos et al. 2010). Although toads may use magnetic cues in some cases (Sinsch 1987; Landler & Gollmann 2011), routes to breeding sites appear to be learned, and navigated mainly via olfactory cues (Sinsch 1987; Ishii et al. 1995).

Despite the remarkable dispersal abilities of cane toads, our current analysis indicates that invasion history does not affect the direction in which an individual chooses to disperse. Toads collected from the western invasion front (and their offspring) follow very straight dispersal paths (Brown et al. 2014), but are as likely to head N, S, or E as they are to head W (current study). Further, the heading at which a field-collected toad disperses does not predict the direction in which its offspring will disperse.

Does this lack of evolution reflect a lack of spatial sorting or natural selection on dispersal direction? Probably not. When the invasion vanguard reached our study site, individual toads did not move in random directions. For the first two years, most toads
dispersed NW; but that bias disappeared over the subsequent eight years. This observation of non-random directionality on the invasion front constitutes clear evidence for spatial sorting by a phenotypic trait – dispersal direction – on the invasion front. The invasion front was dominated by individuals moving NW.

The reason for this non-random directionality of the pioneering toads was a simple consequence of their marked propensity to move in straight paths, combined with the fact that they originated from a common starting point: the apogee of the previous year's invasion, located to the SE of our study site (Phillips et al. 2007). Thus, inevitably, the first toads that arrived were animals that had come from the SE and were heading NW. Even if the colonising source areas contained toads that moved in other directions, none of these would have reached our site because they were spatially sorted by dispersal direction. As time passed, and toads became established beyond our site, some of the SE-dispersing animals passed through our site as well – thus eliminating the initial directional bias.

Despite this evidence for spatial sorting, and the strong theoretical prediction that such sorting can lead to evolutionary shifts, we did not observe any evidence for evolution of dispersal direction. Toads that had been translocated from other sites prior to tracking showed no specific preference to head in a westerly direction, nor in any other consistent direction and no significant trend to move in the direction of their sites of collection. If toads at the invasion front had evolved to follow specific compass headings, we would expect to see westerly dispersal in invasion-front animals but not toads from other populations, but this pattern was not apparent in our data. Invasion-front (Timber Creek) toads moved in random directions when relocated to Middle Point for radio-tracking (probability of a non-random direction, $P = 0.86$). Despite the apparent lack of directionality among toads captured from Timber Creek, if we were located wet of Timber Creek when the invasion vanguard arrived, it would appear to us that all the toads were heading in a common direction – westwards – simply because all the
animals we observe have come from the east. Among toads locally produced in Timber Creek, we would expect as many to disperse eastwards as westwards. Why do we not see the evolution of directionality, despite observing the obvious sorting of individuals by dispersal direction in the first few years of the invasion? The only remaining reason is simply that, in this species, directionality is not heritable, and comparison of parents and their offspring confirms this. Unlike other aspects of movement in this species, such as overall dispersal rate (Phillips *et al.* 2010a) and path straightness (Brown *et al.* 2014), dispersal directions of adults were uncorrelated with dispersal directions of their progeny. This result suggests that directionality has little or no genetic basis in cane toads, so selective forces acting on directionality will not lead to an evolutionary shift.

Directionality does have a genetic basis in other species (Pulido 2007; Liedvogel, Åkesson & Bensch 2011; Penteriani & Delgado 2011; Larranaga *et al.* 2013). Among migratory birds for example, migration directions of offspring resemble those of their parents (Berthold 1991; Helbig *et al.* 1994; Delmore & Irwin 2014). This pattern does not hold in all species, however (Veen *et al.* 2007). In Blackcaps (*Sylvia atricapilla*), the heritable nature of migration direction meant that when a critical mass of novel variants arose, they formed a new overwintering population (in Britain) whose individuals all undertook a novel migratory path across Europe (Berthold *et al.* 1992). This situation of spatial sorting in Blackcaps is analogous to our expectations for toads. If directionality had been heritable in toads, then >70 years of spatial sorting on a westward-heading invasion front should have meant that only westward-heading genotypes were left on the invasion front. Our expectation in this case was not met because the trait – dispersal direction – is not heritable.

Understanding how the directionality of animal movement responds to evolutionary process is not relevant only to biological invasions. Interest in the genetic control of migration and dispersal traits has increased recently due to concern over the effects of climate change on
animal movement (Root et al. 2003; Thomas et al. 2004; Gienapp et al. 2008; Visser 2008). The rate at which such traits can evolve in response to rapid climate change depends strongly on their levels of heritability. While the heritability of dispersal is unknown for the vast majority of taxa, for toads, at least, it is abundantly clear that it is very low, if not zero.

In summary, cane toads at an invasion front showed significant, but transient, directionality in their movement. This directionality arose through a tendency of frontal toads to move in straight paths, combined with their common point of departure (which lay to the SE of our study site). This observation constitutes evidence for spatial sorting by dispersal direction; a situation that, if the trait is heritable, should lead to rapid evolution of dispersal direction on the invasion front. Studies on translocated toads and their progeny, however, showed no evidence for the evolution of directionality: there was no westerly-biased movement of toads originating close to the western invasion front. These results serve as a potent reminder that even the strongest selection and sorting will not lead to evolution unless the trait in question is heritable.

Acknowledgements

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References


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Table 1. Circular statistics and tests of directionality for 257 cane toads tracked at Beatrice Hill Farm over a 10-year period, beginning with the initial arrival of invasive toads. Data are grouped into two-year periods. The Hotelling's test evaluates whether the grand mean heading is non-random, with $P$-values <0.05 indicating significant overall directionality in movement shown in bold font.

<table>
<thead>
<tr>
<th>Period</th>
<th>$N$</th>
<th>Mean heading*</th>
<th>Circular variance</th>
<th>Hotelling's $F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-6</td>
<td>37</td>
<td>345.9°</td>
<td>a</td>
<td>0.61</td>
<td>25.487</td>
</tr>
<tr>
<td>2007-8</td>
<td>77</td>
<td>0.7°</td>
<td>ab</td>
<td>0.84</td>
<td>6.896</td>
</tr>
<tr>
<td>2009-10</td>
<td>45</td>
<td>320.9°</td>
<td>bc</td>
<td>0.92</td>
<td>1.188</td>
</tr>
<tr>
<td>2011-12</td>
<td>30</td>
<td>144.3°</td>
<td>c</td>
<td>0.97</td>
<td>0.137</td>
</tr>
<tr>
<td>2013-14</td>
<td>65</td>
<td>268.5°</td>
<td>c</td>
<td>0.95</td>
<td>0.931</td>
</tr>
</tbody>
</table>

* Mean headings with the same letter are not significantly different, based on pairwise Hotelling’s two-sample $F$ tests.
Table 2. Circular statistics and tests of directionality for 82 cane toads collected from four sites that spanned the invaded Australian range. Each toad was radio-tracked over a five-day period at a common site, during the wet season. Hotelling's tests evaluate whether the mean heading is nonrandom. The letter groupings are from pairwise tests comparing whether the mean heading during each period is different from that in each of the other periods.

<table>
<thead>
<tr>
<th>Source</th>
<th>$N$</th>
<th>Mean heading*</th>
<th>Circular variance</th>
<th>Home bearing from study site</th>
<th>Hotelling's F</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairns</td>
<td>23</td>
<td>194.6°</td>
<td>0.88</td>
<td>109°</td>
<td>2.16</td>
<td>0.15</td>
</tr>
<tr>
<td>Normanton</td>
<td>18</td>
<td>96.0°</td>
<td>0.91</td>
<td>120°</td>
<td>0.53</td>
<td>0.60</td>
</tr>
<tr>
<td>Borroloola</td>
<td>22</td>
<td>88.6°</td>
<td>0.91</td>
<td>125°</td>
<td>1.51</td>
<td>0.25</td>
</tr>
<tr>
<td>Timber Creek</td>
<td>19</td>
<td>216.3°</td>
<td>0.94</td>
<td>194°</td>
<td>0.15</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* Mean headings with the same letter are not significantly different, based on pairwise Hotelling's two-sample $F$ tests.
Table 3. Circular statistics and tests of directionality for 129 common-garden-raised cane toads produced by parents from a range of sites across the toads’ Australian range. Each toad was radio-tracked over a five-day period at the same site. Data are grouped into mid-parent distance groups based on the average of the distances from Cairns at which each toad’s mother and father were collected. Hotelling’s tests evaluate whether the mean heading is nonrandom. The letter groupings are from pairwise tests comparing whether the mean heading during each period is different from that in each of the other periods.

<table>
<thead>
<tr>
<th>Mid-parent distance group</th>
<th>Mean heading*</th>
<th>Circular variance</th>
<th>Hotelling’s F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>267.7° ac</td>
<td>0.88</td>
<td>2.26</td>
<td>0.13</td>
</tr>
<tr>
<td>500</td>
<td>11.18° b</td>
<td>0.99</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>1000</td>
<td>314.5° bc</td>
<td>0.93</td>
<td>1.43</td>
<td>0.25</td>
</tr>
<tr>
<td>&gt;1500</td>
<td>281.0° bc</td>
<td>0.89</td>
<td>0.50</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* Mean headings with the same letter are not significantly different, based on pairwise Hotelling’s two-sample F tests.
**Figure legends**

**Fig. 1.** Map showing the location of four collection sites across the cane toads’ range across northern Australia: Cairns QLD (16.9°S, 145.8°E), Normanton QLD (17.7°S, 141.1°E), Borroloola NT (16.1°S, 136.3°E), and Timber Creek NT (15.7°S, 130.5°E). The asterisk indicates the location of the research station where the radio-tracking study took place.

**Fig. 2.** Circular distributions of mean headings of cane toads radio-tracked at a single site over 10 years. The toads were collected in this area each year after the initial arrival of the toad front. Arrows indicate the direction of the grand mean heading of the sample and the length of the arrow indicates its level of significance. During the first two years of the invasion (a) toads moved at a significant northward heading (346°). During the next two-year period (b) toads still moved with significant directionality, although more variation in headings was observed among individuals (i.e. some toads headed south). During the remaining periods (c, d, e) the samples of toads moved at random headings (i.e. with no significant overall directionality). Sample sizes for each period are 2005-06, \(N = 37\) toads, 2007-08, \(N = 77\), 2009-10, \(N = 45\), 2011-12, \(N = 30\), and 2013-14, \(N = 65\).

**Fig. 3.** Temporal change in variance of movement direction among 257 cane toads radio-tracked over 10 years. Variance was lower during the initial stages of invasion, because most toads were moving in the same direction.

**Fig. 4.** Circular distributions of mean headings of 82 radio-tracked cane toads collected from four sites across the species’ northern Australian range (a) Cairns, \(N = 18\), (b) Normanton, \(N = 23\), (c) Borroloola, \(N = 22\), and (d) Timber Creek, \(N = 19\). Toads were brought to a
common site in the Northern Territory and tracked for five days each. No significant directionality was observed among toads from any of the collection sites. Toads from Borroloola showed an almost-significant tendency ($P = 0.06$) to head towards their original capture site.

**Fig. 5.** Circular distributions of mean headings of 129 common-garden-reared cane toads whose parents had been collected from four sites across the species’ northern Australian range. Parental location was quantified into mid-parent distance groups (calculated as the average of the distances of the parental capture sites from the site of the toads initial introduction, Cairns QLD (a) mid-parent distance = 0 km, $N = 20$, (b) mid-parent distance = 500 km, $N = 41$, (c) mid-parent distance = 1000 km, $N = 57$, and (d) mid-parent distance = 1636 km, $N = 11$. All mid-parent distance groups lacked significant overall directionality in movement.
Fig. 1.
Fig. 2

2005-6

2007-8

2009-10

2011-2

2013-4
Fig. 3

![Circular variance over years](image)
Fig. 4

Cairns

Normanton

Boroloola

Timber Creek
Fig. 5

0 km

500 km

1000 km

1500 km
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