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THE
UNIVERSITY
OF EXETER

AGEING AND PHYSICAL PERFORMANCE IN WILD CRICKETS

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……………………………………………………………..

Summary

Aging and longevity variation have been a hot topic for researchers for many decades. Despite the great number of studies on senescence and longevity, both the causes and the mechanisms of aging remain a subject of debate. Though it can be difficult to study senescence traits in the wild this is seen as vital to understanding the evolution of senescence especially as theory suggests it is so closely related to experienced levels of extrinsic mortality. In this thesis, I use a long-term observation study which utilises 24 hour observational video to capture the lives of a population of field crickets (*Gryllus campestris*) throughout their adult lives. This provides the opportunity to ask a number of questions around the theme of senescence and lifespan. The first section aims to find whether senescence is seen in a trait directly affecting rate of predation and if sex biased predation rates alter this. The second section examines lifespan and the causes of the variation we see within species. My research identifies no senesce or sex biased aging in this wild population. I found that there was selective disappearance of faster individuals in early life. I saw no effect of speed performance on the lifespan of these crickets, but there were trade-offs between various activity measures and lifespan. My work adds to current understanding of how variation in aging rates and life-history traits are maintained under natural selection. My findings underline the fact that this complex subject requires further testing in the natural environment.

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CHAPTER 1

Senescence of fleeing speed in a wild orthopteran (*Gryllus campestris*)

ABSTRACT

The evolution of senescence is greatly influenced by extrinsic mortality and the consequent weakening of selection with increasing age. Despite the fact that senescence is a popular research topic, the knowledge of underlying mechanisms is still limited, as is the effect of natural selection on these mechanisms. The purpose of this study was to ascertain whether there is senescence in a trait putatively associated with the likelihood of predation in a wild population of a field cricket species. We also set out to look at the differences of this trait between males and females, as the study species is known to have gender biased predation rates when living in pairs. Where previous academic work may have been unable to sufficiently measure intrinsic declines affecting predation rate, with exact ages of individuals and age of death this particular study gives a new perspective on senescence in the wild. Using slow motion recording technology, burst speed of tagged and monitored crickets was measured in response to a predator stimulus. We found that burst speed increased with age up to a point at which it leveled off. We found evidence for selective disappearance of faster individuals from the population which at the population level will tend to mask the increase in speed observed within individuals. We were also able to establish that there was no senescence of burst speed seen after this point.

1. INTRODUCTION

1.1 Senescence

Senescence is an apparently inevitable yet curious process that all living things encounter as they travel towards death. Consequently, the topic of aging and senescence has been somewhat of an enigma over the years, one that has captivated a great many researchers (Kirkwood and Austad 2000). In the field of bio-gerontology, senescence is generally accepted to be the failure to replace deteriorating systems. Senescence causes declines in both physiological condition (functional senescence) and fecundity (reproductive senescence), and increases the risk of mortality with age (actuarial senescence) (Williams 1957; Bonsall 2006). Originally the question of why senescence occurs was thought to be unsolvable (Medawar 1952), but is now a well-studied and better explained avenue in biology. Despite this, the underlying mechanisms of senescence still predominantly need to be identified and how natural selection acts on these mechanisms remains, for now, slightly mysterious (Holliday 2006; Hayflick 2007).

1.2 Evolution of senescence

The past decades have thrown out a number of theories, each striving to explain why senescence, a detrimental process, can persist when natural selection is committed to removing such things from populations. Evolutionary theories of senescence are centred around variation in the strength of natural selection throughout life (Archer and Hunt 2015). The strength of natural selection on any particular trait decreases as the individual gets older. This is because of the continual risk of mortality and the declining likelihood of survival and so reproduction, this is true even without the occurrence of senescence (Williams et al. 2006).

If reproduction occurs before a trait has been expressed, the strength of natural selection on that trait will be greatly reduced. This means that traits expressed in early life will have a greater effect on fitness than those expressed in later life. Potentially detrimental later acting genes will be passed on allowing the accumulation of deleterious mutations acting later in life. This explanation for senescence is known as the mutation accumulation theory (Medawar 1952). The antagonistic pleiotropy theory builds on this, suggesting that beneficial early acting alleles will be strongly selected for even if those same alleles are detrimental later in life, as their detrimental effects are under weaker selection than their positive effects, inadvertently selecting for senescence (Williams 1957).

The disposable soma theory is a mechanistic implementation of antagonistic pleiotropy, explaining that investment of limited resources in reproduction early in life is at the expense of maintenance later in life. Fundamentally, the theory describes a trade-off between reproduction and survival, preferentially allocating energy to either reproduction or somatic maintenance (Kirkwood 1977). In light of these theories we can start to understand how senescence can continue to escape from the clutches of natural selection.

Unfortunately, our current comprehension of the causes of senescence leaves us lacking in sufficient knowledge to reliably explain the variation in the onset of senescence and longevity both among and within populations (Nussey et al.

2008; Jones et al. 2014). In consideration of this, research efforts have been focusing more recently on causes of variation seen in wild populations (Partridge and Gems 2007; Monaghan et al. 2008; Wilson et al. 2008).

1.3 Extrinsic mortality

As previously mentioned, extrinsic mortality causes weakening of natural selection over life leading to senescence. Due to the complexity of natural populations there are evidently numerous environmental pressures that alter an individual's likelihood of survival. When one takes into account the intrinsic condition of any given organism, it quickly becomes apparent that not all members of the population are equally as susceptible to extrinsic mortality. We may therefore consider that intrinsic condition could have an effect on the evolution of senescence.

Condition dependent extrinsic mortality could in part explain the variation seen in senescence both among and within populations (Chen and Maklakov 2012). Presuming that mortality is condition dependent it would be expected that those individuals with better capacity for evading the cause of mortality would survive for longer. Over time that would lead to delayed or reduced senescence in those traits associated with avoiding the hazard (Williams et al. 2003).

If condition changes with age, condition dependent mortality could also, therefore, be age dependent mortality (Williams et al. 2006). In this scenario, deaths in later adulthood are a consequence of interactions between intrinsic declines and extrinsic pressures. The degree of influence of extrinsic mortality on the evolution of senescence is potentially determined by the extent to which mortality is condition dependent or not (Chen and Maklakov 2012). The implications of condition dependent mortality can therefore have complex effects on senescence. Due to both changes in extrinsic and intrinsic mortality causes there could be earlier or later onset of senescence, or no change at all (Reznick et al. 2004), a reason why natural studies could be so important in this field.

1.4 Laboratory vs natural studies

Research from both laboratory and wild studies are imperative in understanding senescence, though each technique has a somewhat different aim and outcome. Laboratory studies have taught us a great deal about the mechanisms behind senescence and its intrinsic effects (Nussey et al. 2008). Despite this, the field of senescence is still in its infancy and we have only touched the surface of understanding how senescence works. It is indisputable that these intrinsic senescent declines can have an impact on the likelihood of extrinsic mortality (Koopman et al. 2015). Yet the reason for such variation in senescence still requires more research. It becomes clear that we must use wild studies to test evolutionary theories and provide the finer details of the ecological importance of these senescent declines (Kirkwood and Austad 2000; Williams et al. 2006; Monaghan et al. 2008; Nussey et al. 2008).

1.5 Importance of natural studies

In the light of the evolutionary theories explaining senescence, it was initially believed that it would seldom be seen in the wild. It was assumed that high levels of extrinsic mortality would remove individuals before they showed signs of deterioration (Medawar 1952; Kirkwood and Austad 2000). Recent work has focused precisely on this, showing that senescence is almost ubiquitous across taxa in the wild, including: large mammals, birds, reptiles, fish and even flies with an adult lifespan of just a few days (Bonduriansky and Brassil 2002; Reznick et al. 2004; Brommer et al. 2007; Massot et al. 2011; Marescot et al. 2015).

It is vital to look to nature to gain a better understanding of the evolution of senescence, and causes for great variation. This is particularly important as some recent studies demonstrate that natural populations don't always conform to the simplest versions of these evolutionary theories. For example, studies of the response to rate of extrinsic mortality on the evolution of senescence in the wild (e.g. Reznick et al. 2004; Walsh et al. 2014). Indeed, there appear to be contradictions between both studies in the wild and the predictions of theory, and between studies in the wild and findings from laboratory populations (e.g. Stearns et al. 2000; Chen and Maklakov 2012).

1.6 Measuring senescence in wild populations

When studying actuarial senescence, mortality is measured throughout life. Many studies working with wild populations use capture-mark recapture/re-sight methods (Loison et al. 1999; Péron et al. 2010; Sherratt et al. 2010; Marescot et al. 2015). This allows an estimation of lifespan and rate of baseline mortality (a combination of extrinsic and intrinsic mortality) in a population. It can be difficult to differentiate between permanent dispersal and death, but this is necessary to avoid bias. Using a combination of knowledge of the study population's dispersal habits, and recovery of deceased tagged individuals it should be possible to gauge an accurate mortality rate (e.g. Boonekamp et al. 2014). It is also occasionally possible to determine cause of mortality, yet this very feature is likely to affect the probability of finding an individual after death. For this reason using data such as this to estimate mortality in a population can be biased. Moreover this is typically only possible with large species (Schaub and Pradel 2004; Péron et al. 2010; Marescot et al. 2015).

It can be very difficult to gauge intrinsic condition in wild animals; however, there are a number of studies that have examined the physical decline of various fitness components in the wild. Some look at reproductive output, to see if this shows signs of senescence over lifetime. Reproductive senescence has been seen in natural populations of moose, sheep and lizards, to name a few, with the reproductive output decreasing by both litter size and offspring survival. Those individuals with the best intrinsic condition are able to invest the most in reproduction (Berube et al. 1999; Ericsson et al. 2001; Massot et al. 2011).

Body mass has been used as a measure of intrinsic condition and is seen to decline in wild populations with age (Nussey et al. 2011; Tafani et al. 2013; Hämäläinen et al. 2014). It is related to physiological performance, and so can give a fairly reliable indicator of intrinsic condition. Physiological performance itself is a valuable indicator of intrinsic condition, but one that can prove difficult to measure in the wild. Hämäläinen et al. (2015) measured strength and dexterity required for food acquisition in a small primate. This measure of strength could therefore be used to estimate likelihood of survival and longevity. This focus on intrinsic declines in traits that affect survival will enable a greater appreciation of the effects that senescence has on fitness (Bouwhuis et al. 2012).

1.7 Measuring intrinsic decline and functional senescence

Studying wild individuals from ‘cradle to grave’ is challenging, and is perhaps the main reason why laboratory studies dominate the research in the field of senescence. In the wild, high extrinsic mortality causes populations to have considerably fewer individuals in higher age classes which can complicate data analysis across lifespan (Nussey et al. 2008). However, a major difficulty can be disentangling these intrinsic declines from demographic patterns.

When looking at senescence in an age structured population, changes within and among individuals often differ. However, senescent declines in performance within individuals can be masked by selective disappearance of fast senescing individuals from the population. The remaining population consequently has a higher proportion of high condition individuals. It is crucial therefore, to separate the within individual patterns from cross-sectional patterns, to draw apart the processes causing senescence (van de Pol and Verhulst 2006).

1.8 Crickets fleeing speed an indicator of senescence

The field cricket *Gryllus campestris* lives in and around burrows excavated among the grass in meadows or other grassy habitats. This species experiences high predation by birds, mammals and invertebrates, which can be avoided by quickly running into their burrow (Rodríguez-Muñoz et al. 2011). It is clear that the time it takes a cricket to run into its burrow is likely to be a key determinant of the likelihood of surviving an attack, and hence, being faster is expected to increase the chances of survival (Rodríguez-Muñoz et al. 2011;

Niemelä et al. 2015). Speed is also a potential measure of intrinsic condition. There is a known sex bias in predation in this species, where a pair of crickets occupy a single burrow, male predation rates increases massively relative to female predation rates. The predation rate is different due to males apparently allowing females priority access to the burrow (Rodríguez-Muñoz et al. 2011). We monitored the lives of a wild population of individually tagged adult *G. campestris*, and measured fleeing speed at several points over their lives.

According to evolutionary theories one would expect to see the effects of senescence in crickets in relation to extrinsic mortality risks which will create decreasing strength of selection with increasing age. As males and females are subject to different levels of extrinsic mortality we expect to see different rates of senescence. If there are high levels of condition dependent extrinsic mortality we would expect to see a greater effect of selective disappearance in males rather than females (Hämäläinen et al. 2014). Due to shifting resource investment over life, there will be differing levels of selective disappearance, but similarly each trait will be differently susceptible to this selective disappearance (Zhang et al. 2015).

We would also expect to see an influence of temperature on the speed of crickets, as they are ectothermic. Speed is expected to increase with temperature up to some threshold (optimum performance temperature). We might expect this threshold to be higher than the temperatures experienced during our study. At higher temperatures we would predict that crickets move faster, as has been seen in other insects. A specific temperature must be reached to warm muscles enough to enable movement (as found for example in flight muscles of *D. melanogaster*). It is likely, therefore, that temperature will be important for the 'jumping' movement of crickets (Lehmann 1999; O'Neill and Rolston 2007; Norris and Kunz 2012). The distance of the cricket from its burrow is also likely to affect the probability of predator attack and success. Predators may select targets that are more vulnerable, or may find crickets easier to spot further from their burrows. There may be some effect of distance from the burrow on cricket speed, with crickets either investing more in speed, or perhaps reaching higher speeds with a greater distance to accelerate (Hiraguchi and Yamaguchi 2000).

In this study we aim to determine whether there is senescence in fleeing speed, a trait which we expect to directly influence the likelihood of predation. We will also compare the rate of senescence between males and females in a population with a known gender-bias in predation rates.

2. METHODOLOGY

2.1 Study species

Gryllus campestris is a field cricket species found living in small burrows in meadows and other grassy areas. Although a threatened species in its northern range, this cricket is fairly common in Central and Southern Europe (Kenyeres 2006; Hochkirch et al. 2008).

This particular cricket has a heterodynamic lifestyle, meaning it has an overwintering stage of dormancy during its life. *G. campestris* digs a burrow in the ground and overwinters as a late instar nymph emerging the following year. In our study site in Northern Spain, adults emerge between late April and May

(Fisher et al. 2015). The fact that they live in close proximity to their burrows makes it possible to monitor the majority of adult life for each individual (Rodriguez-Munoz et al. 2010).

G. campestris are univoltine, producing a single generation each year (Masaki and Walker 1987). This makes it a suitable species for the study of senescence in the wild, as they can be thoroughly studied over the course of a season covering their last nymphal stages through to adulthood. The vast majority of previous studies on crickets also make them ideal for studying senescence, with the potential to combine evolutionary theories of senescence with others such as sexual selection (Archer and Hunt 2015).

2.2 Study site

This study was carried out at the “WildCrickets” field site in Asturias; Northern Spain (Rodriguez-Munoz et al. 2010). Data were collected between May and June 2015. By this time all the individuals occupying the meadow had reached adulthood.

2.3 Experimental design

As a part of the wider research of the “WildCrickets” project the population of crickets is monitored continually throughout the reproductive season using a network of infra-red equipped CCTV cameras (iCatcher ver. 6.0, www.icode.co.uk/icatcher). From mid-February the meadow is thoroughly checked for cricket burrows on a weekly basis, each burrow is given a flag with an individual number and cameras are placed at up to 140 burrows. When a cricket is observed emerging as an adult, it is captured and tagged within 2-4 days. Each cricket is given an individual tag which is glued to its pronotum (Fisher et al. 2015). Individual tagging provides a unique opportunity to look at within and among individual differences. Each individual is weighed, measured and has a haemolymph sample taken for DNA profiling. The 24 hour video recording also provides the exact time and date of emergence to adulthood and adult lifespan for most of the crickets included in this study (Rodriguez-Munoz et al. 2010).

We measured ‘burst speed’ of a cricket’s retreat or fleeing response (here-after referred to as BS) after a ‘scare’ stimulus had been deployed, during the 2015 field season. This was repeated daily to gather a dataset of BS throughout the first 35 days of the lives of these field crickets (mean lifespan ± standard error for 2015 was 28 ± 1 day).

To measure the BS of *G. campestris*, we used three compact digital cameras (Fujifilm FinePix XP70) capable of slow-motion recording. Footage was taken at 240 frames per second (fps) to produce a highly detailed recording of each run. The height of the camera was important, too close and the cricket would startle from the trigger mechanism, too far and the quality was compromised. Each camera was set on a tripod keeping it approximately 15cm above the ground. At each measuring event the camera was placed above the burrow with the burrow number in shot. This simplified data collection in the field, being able to collect this information from the video, burrow temperature was the only other variable that needed recording. Temperature was recorded using a portable thermometer with a probe that was placed on the ground approximately 5cm from the burrow entrance.

The cameras required an external remote controlled trigger system which would start recording from a distance of up to 30m. This avoided any disturbance to the crickets by walking near the burrows during data collection. This would also ensure that all scare stimuli would be from the same source.

G. campestris are particularly sensitive to vibration which they use as the main cue for predator flight response (Niemelä et al. 2015).The predator mimicking device was simply a miniature remote control car (8cm long x 3.5cm width) released through a plastic tube (25cm long), strapped to the side of the tripod. This allowed the stimulus to be deployed from a constant distance and ensured that the car struck the ground approximately 20cm from the burrow, having fallen from a constant height.

A measuring session for each cricket aimed to record 3 vibration simulated running events in a row. These repeat measures allow an average daily BS score to be calculated for each cricket to reduce noise in the data. The camera and predator mimicking device were placed above an active burrow. A shade was also placed over the burrow a few minutes prior to the stimulus to prevent any effects of direct sunlight hitting these ectothermic invertebrates. A study of the closely related *Gryllus bimaculatus* demonstrated that the body temperature of a cricket under an artificial light source falls to that of room temperature within 1 minute of it being switched off (Remmert 1985). We would therefore expect *G. campestris* to heat up rapidly in the sun and cool down once inside their burrows or under the shade. This shade and subsequent control of the range of temperatures crickets are exposed to was necessary as direct sunlight could alter both their capacity to move and their behaviour (O’Neill and Rolston 2007; Norris and Kunz 2012).

The cricket was left to emerge from the burrow and for the temperature probe to stabilise for 2-3 minutes. The camera trigger was then set off and the ‘predator’ deployed shortly afterwards. The car was then placed back to the top of the tube and after a wait of 1-5 minutes (time taken for the cricket to remerge) the process was then repeated. 3 cameras were used simultaneously to optimise data collection, each placed at least 2m from another camera to prevent unintentional disturbance.

In total, 71 individual *G. campestris* specimens were included in the data collection. Measurements were taken strategically, starting from burrow number 1 and working through the burrows in ascending order, measuring any crickets seen. Both nymphs and adults were measured. Subsequently a list of those crickets that had been measured and those that hadn’t was composed, allowing for measurement of different individuals over time. Wherever possible, each cricket had its BS measured every 1-5 days. Information on occupancy of burrows was compiled daily from the observational videos, accounting for each cricket and burrow number.

2.4 Data collection

Over the course of 26 days between 02/05/2015 and 04/06/2015 we recorded 771 videos from which it was possible to measure the BS of crickets responding to our stimulus. It was only possible to record videos on days where crickets could be seen outside the burrow, this excluded days with continuous heavy rain. On average, each cricket was recorded on 4 separate days (SD ± 3 days), ranging between 1 and 13 recordings per individual. Recordings were made of both nymphs and adults including those aged 0-35days. As *G. campestris* are active most of the day, videos were taken between the hours of 11:00 and 17:00 when the highest levels of activity were observed. Crickets were less active before and after this time, most likely due to the lower temperatures.

Videos were watched using Avidemux software (www.avidemux.en.softonic.com) as it allows viewing the video frame by frame. BS of the cricket was measured by how many pixels were travelled on screen over the first 10 frames, using an onscreen measuring tool ‘small measure’ (www.donationcoder.com/Software/Skrommel/index. html#SmallMeasure). At the beginning of each video the burrow number (4mm high) was measured on screen (in pixels) to later allow conversion from pixels into mm. We noted whether the cricket was facing into the burrow or facing out. Those crickets facing in had to make no adjustments to trajectory before running for the burrow. If a cricket had to turn greater than approximately 90° before running it was classed as facing out of the burrow. The exact time the cricket started moving was recorded and BS was measured from the point at which the cricket was running straight towards the burrow. If the cricket was facing out of the burrow measurement of BS started as soon as it had finished turning. To measure the BS the measuring cursor was placed at any easily visible point on the cricket and then the frames were advanced, the measure was then placed in the same position on the cricket giving a reading of the exact number of pixels moved over ten frames. The time was then recorded again when the cricket had either stopped moving or was fully hidden.

2.5 Statistical analysis

All statistical analyses were carried out using R v. 3.2.1, and R package lme4 (Bates et al. 2014) was used to carry out the linear mixed effects models.

2.5.1 Senescence of burst speed

Linear mixed effects models were used to assess whether BS showed signs of senescence, by decreasing over lifetime. The BS measure used was that of the first 10 frames of video, as the measure of BS at this point will be similar for all crickets (going from stationary to quick movement), and it is this short burst of speed we are interested in. All analysis used this measure of BS unless stated otherwise. We partitioned age into mean age and ∆age to separate among from within individual patterns (van de Pol and Verhulst 2006). Mean age was used as a measure of age effects among individuals, calculated as the mean of all the ages at which each cricket was observed. ∆age was used as a measure of age effects within individuals; this was determined by taking difference between the cricket's age on a given day and its average age over the period we monitored it (age= average age + ∆age). For all linear mixed effects models in this study, the model comparison Chi-square approach was used adding terms to the model and using ANOVA to test for a significant improvement of fit.

2.5.2 Distance, temperature, sex and burst speed

To look at the effects of other variables on the BS of crickets a linear mixed effects model was used. Fixed effects included in the final model were: distance from the burrow; burrow temperature; and the sex of the cricket. Individual identity nested within day measured was a random effect.

2.5.3 Variation in speed over one escape event

To assess how the cricket accelerates whilst running into the burrow a one-way analysis of variance (ANOVA) was used. This compared the first three sections of the run, split into sections of 10 frames (first 10 frames, second 10 frames and third 10 frames). Runs with fewer than 3 sections were omitted from this analysis.

2.5.4 Repeatability

To test the repeatability of a cricket's BS within a day, a mixed effects linear model was used with tag-day as the random variable. The repeatability estimate was then calculated looking specifically at the proportion of total variance that is explained within individuals on a given day, assessing statistical significance using credible intervals (as demonstrated in Nakagawa and Schielzeth (2010)). Finding this measure of repeatability is important, because if BS is totally erratic (not repeatable within a day) we are unlikely to be able to assess whether it is repeatable or changes systematically over the lifetime.

3. RESULTS

Means are presented with standard errors unless stated otherwise

3.1 Senescence of burst speed

3.1.1 Complete-life analysis

In total 71 crickets (33 females and 38 males) were included in the study with ages ranging from 0-35 days post adulthood and a mean lifespan of 29 ± 0.5days. The mean BS recorded for the first 10 frames was 228.96 ± 4.29mm/s. Both sexes were measured on average 1.7 times per recording day.

The mixed linear effects model used to test the effects of age on BS included the variables; average age; Δage; age^2; burrow temperature; sex and interactions of sex as fixed effects. Age^2 tests for any quadratic effect of age on BS. Cricket tag (individual identity) was a random effect, this controlled for the multiple measures for each individual within and among days. Sex and interactions of sex were not included in the final model.

A quadratic relationship between BS and age was indicated by the model (*p*<0.001). This reveals an increase in BS in early adulthood and a decrease towards the end of life (Fig.1, Table1).

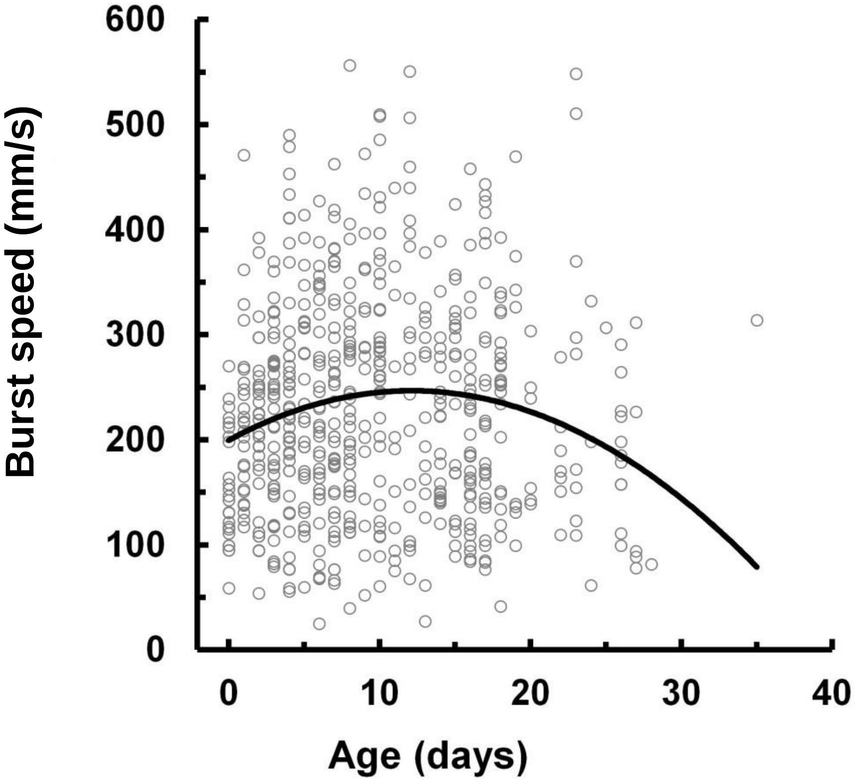

Figure 1. This curve was predicted from the mixed linear effects model and shows that there is a quadratic effect of within individual (delta) age on speed, speed increases early in life and declines in later life.

Table1. Results of the ANOVA comparing different models analysing the effect of age on burst speed. We selected the model with the smallest AIC (marked in bold). Individual is a random factor. Because the selected model has a quadratic fit, we calculated the maximum of the quadratic function (peak performance) and split the dataset into pre-peak and post-peak subsets These subsets were analysed separately dividing age into within subjects (DeltaAge) and among subjects (MeanAge) components in order to examine potential selective loss or gain effects (following the approach of (van de Pol and Wright 2009). The lower table shows the results of the separate analyses based on the selected model using *lmer*. Coefficients with significant *P* values are highlighted in bold italics.

MODEL	AIC	BIC	logLik	deviance	P
Burst speed					
Individual + Age	7182.4	7204.3	- 3586.2	7172.4	
Individual + Temperature + Age	7170.3	7196.7	- 3579.2	7158.3	***< 0.001***
Individual + Temperature + Age + Age^2	**7157.6**	7188.3	- 3571.8	7143.6	***< 0.001***
Individual + Temperature + Age + Age^2 + Sex	7159.1	7194.2	- 3571.5	7143.1	0.469
Individual + Temperature + Age + Age^2 * Sex	7160.8	7200.3	- 3571.4	7142.8	0.626
Burst speed pre-peak					
Individual + Age	4855.5	4879.5	- 2421.8	4843.5	
Individual + Temperature + Age	**4851.5**	4879.5	- 2418.8	4837.5	***0.014***
Individual + Temperature + Age + Sex	4853.1	4885.1	- 2418.6	4837.1	0.535
Individual + Temperature + Age * Sex	4851.9	4887.8	- 2416.9	4833.9	0.070
Burst speed post-peak					
Individual + Age	**2302.2**	2321.7	- 1145.1	2290.2	
Individual + Temperature + Age	2302.5	2325.2	- 1144.2	2288.5	0.189
Individual + Temperature + Age + Sex	2304.4	2330.3	- 1144.2	2288.4	0.728
Individual + Temperature + Age * Sex	2304.8	2334.1	- 1143.4	2286.8	0.216

	Burst speed pre-peak			**Burst speed post-peak**		
Fixed effects	**Estimate**	**SD**	**P**	**Estimate**	**SD**	**P**
Intercept	74.243	66.08	0.262	215.699	45.16	***< 0.001***
Temperature	7.176	2.914	***0.014***			
Within individual	9.351	1.735	***< 0.001***	- 2.866	3.048	0.351
Among individuals	1.875	3.285	0.570	0.322	2.506	0.988
Within vs. among individuals	- 7.475	3.746	***0.048***	3.188	3.942	0.421
Random effects	**Variance**	**SD**	**N**	**Variance**	**SD**	**N**
Individual	1930.0	43.9	65	141.0	11.9	47
Residual	8550.0	92.5		9485.7	97.4	

As the analyses revealed a quadratic effect of age, we wished to examine this relationship further. Age was subset into pre- and post-peak performance at the highest point of the age curve to assess significance as individual linear terms with younger individuals in the first half and older individuals in the second. The highest performance of BS was found at age: 12.11 days so age was split into pre-peak (<12.11 days) and post-peak (≥12.11 days).

3.1.2 Pre-peak analysis

There were 64 individuals included in the pre-peak analyses, 31 females and 33 males.

Pre-peak burst speed performance

The pre-peak model included average age, ∆age, burrow temperature, sex and interactions of sex as fixed effects; cricket tag was a random effect. Sex and interactions of sex were not included in the final model. The age of crickets in the pre-peak analysis ranges from 0 to 12.10. There is a significant linear increase in BS performance of crickets over age pre-peak p=0.014 (Fig.2, Table1). Crickets are therefore seen to speed up after emergence to adulthood. The significant interaction between the within and among individual effects (van de Pol and Wright 2009) identified in the final model (p=0.048) indicates a difference in the slope of the within and among individual effects on BS. Comparing these slopes (Fig.2) reveals that although individuals increase in speed as they age over the first 12 days of their adult lives, the mean speed of the population fails to increase. ∆age and average age data were all mean centred so that both age terms can be easily viewed on the same graphs.

The likely explanation for the difference in within and among individual patterns is that there is selective disappearance (van de Pol and Wright 2009). Up until the age at which they are fastest, faster crickets are seemingly lost from the population more frequently than slower ones. There was no significant effect of sex or interactions affecting BS pre-peak.

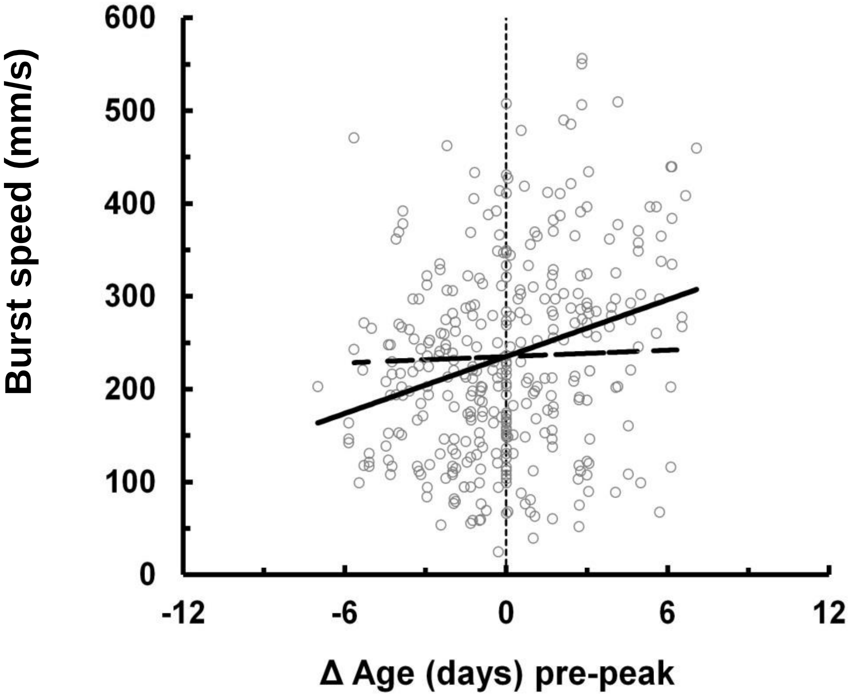

Figure 2. These lines represent predicted values from mixed linear effects model of pre-peak speed (split by age) with delta (solid line) and mean (dashed line) age. Speed increases with age and the interaction between within individual (delta) and between individual (mean) ages suggests selective disappearance.

3.1.3 Post-peak analysis

There were 47 individuals included in the post-peak analyses, 18 females and 29 males.

The model for post-peak included average age, ∆age, burrow temperature, sex and interactions of sex as fixed effects, cricket identity was a random effect. The age of crickets in post-peak analysis ranged from 12.11 to 35. The BS of crickets post-peak was not seen to significantly decline with age (Fig.3, Table1).

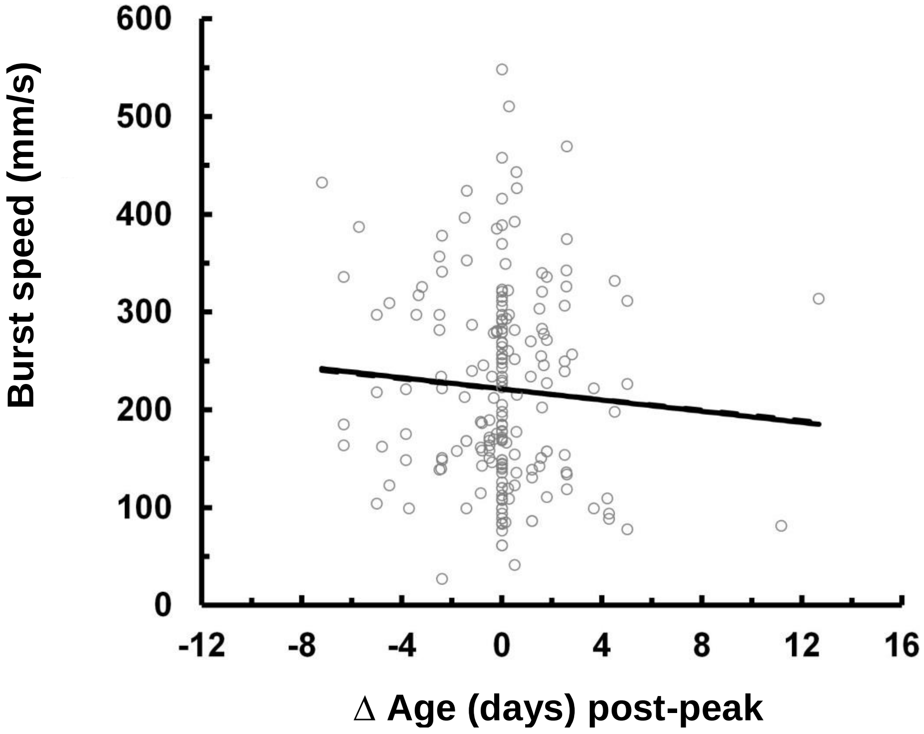

Figure 3. These lines represent predicted values from mixed linear effects model of post-peak speed (split by age) with delta age (solid line) and average age (dashed line). Change in speed is not significantly affected by age.

3.1.4 Distance, temperature, sex and burst speed

The final model included distance and burrow temperature as fixed effects with cricket tag nested within day measured as a random effect. The average distance a cricket was from its burrow was 20.48 ± 0.42mm (Female: 19.63 ± 0.04mm, Male: 21.06 ± 0.03mm). Crickets ran faster the further they were from the burrow entrance (lme: X_1^8= 4.65, *p*=0.03) (Fig.4.). There was an interaction between distance and sex showing that at a greater distance from the burrow females move faster than males.

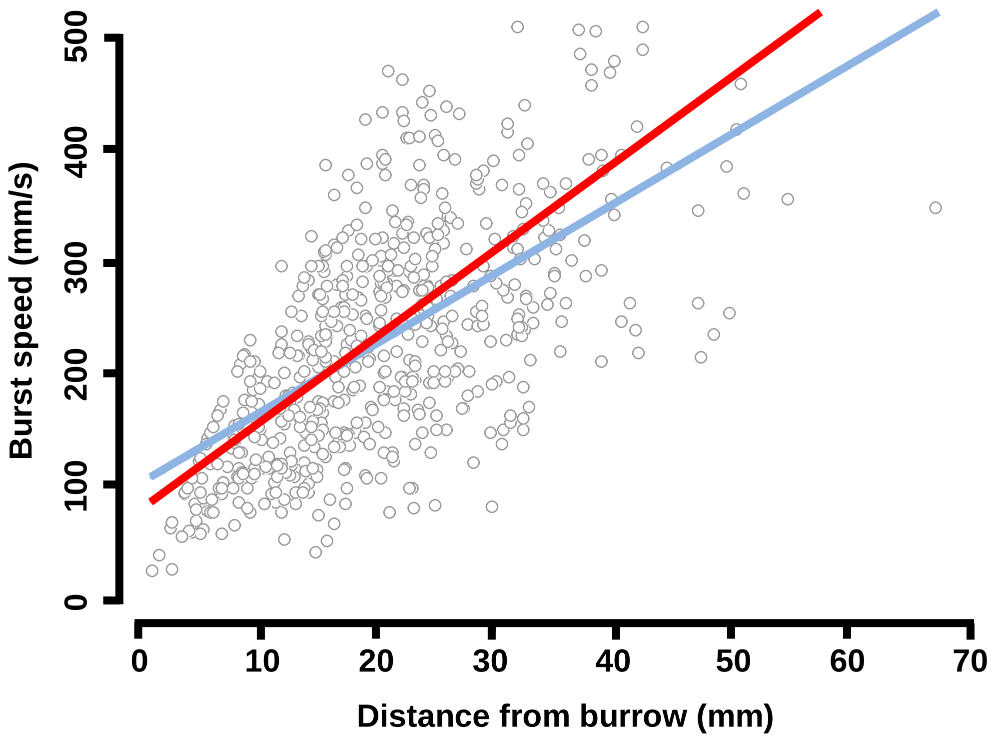

Figure 4. These lines represent predicted values from mixed linear effects model of burst speed with increasing distance from the burrow. There was an interaction of sex (blue line- male, red line- female) suggesting females are faster the further from the burrow.

The average temperature over all the recordings ranged from 16.3°C to 28.7°C with a mean of 20.86 ± 0.08°C. *G. campestris* move faster at higher temperatures (lme: X_1^6= 9.45, *p*=0.002) (Fig.5.), as would be expected of an ectotherm.

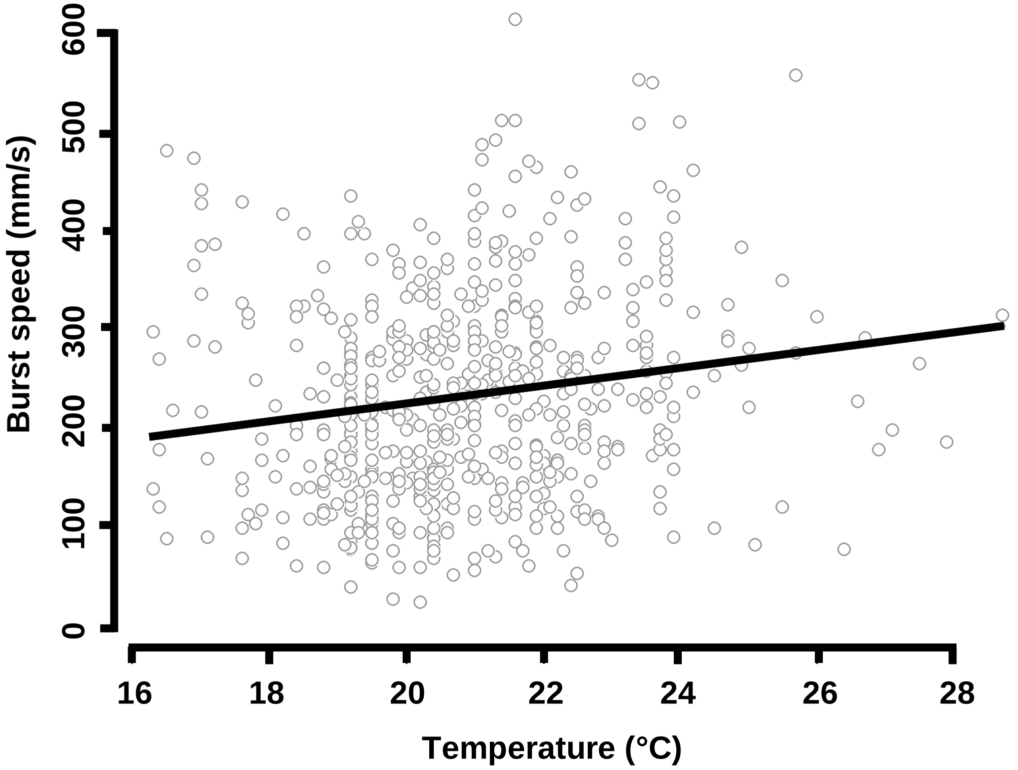

Figure 5. This line has been predicted from the linear mixed effects model and shows that as temperature increases crickets are seen to move faster.

3.1.5 Variation in burst speed over one escape event

Crickets slow down as they approach the burrow; after 121ms there was a 55% decrease in BS. Speed of first (237.300 ± 6.066mm/s), second (177.079 ± 5.893mm/s) and third (105.919 ± 4.087mm/s) 10 frame sections of recording illustrate this effect, (one-way ANOVA, $F_{2,543}$= 16.59, p<0.0001) (Fig.6). It seems that crickets produce a burst of speed at the beginning of a run and slow down as they reach the cover of the shelter.

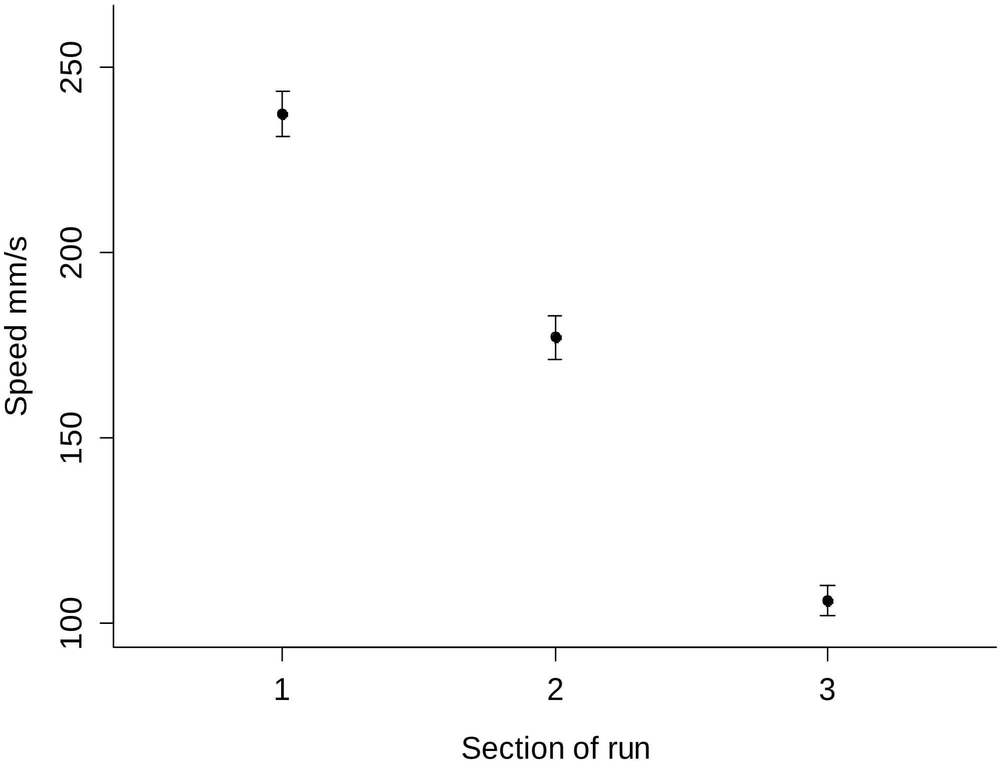

Figure 6. Mean speed of first three, ten frame sections ± SEM.

3.1.6 Repeatability

Using the explained variance from individual cricket on an individual day and the residual variance from the linear mixed effects model, the repeatability of BS within crickets within a day was found to be 0.17 (97.5%CI, LCI=0.09, UCI=0.35). As the confidence interval measures are narrow and do not cross zero this suggests that there is repeatability of BS within individuals within a day.

4. DISCUSSION

Using our observations of BS over the lifetime of *G. campestris* we were able to look at patterns of senescence and discern effects of selective disappearance. We found that BS increases over the first 12 days of adulthood within individuals, but not in the population as a whole, this is due to selective elimination of faster individuals. We have also been able to confirm that there is no senescence or selective disappearance seen in relation to BS in later life.

4.1 Change of burst speed Pre-peak

The increase in BS pre-peak is not consistent with the prediction that extrinsic mortality will cause slower individuals to be predated at a higher rate. We have seen, rather unexpectedly, that crickets speed up over the first 12 days of their adult lives and that the fastest individuals appear to be selectively lost from the population, which as a whole does not increase in BS. We predicted that slower individuals would be more likely to suffer from predation, this looks not to be the case. On the contrary our findings suggest that the faster individuals experience a cost of being able to run fast (or of some linked trait), which leads to them dying earlier. What these costs might be is not obvious. Mortality this early in adulthood is likely to be dominated by predation, suggesting that faster individuals may differ in their behavior from slower ones in such a fashion that they expose themselves to a greater risk of predation, for instance by being generally more active outside their burrows.

We have seen that BS is closely linked to distance from the burrow, the further from the burrow the faster the individual is. It has been previously shown that distance from the burrow can be used as a proxy for boldness in *G. campestris*

(Niemelä et al. 2015). It is feasible that faster crickets are also spending more time out of the burrow and/or further from the burrow, leaving them more vulnerable to predation. Though they have a higher BS, bolder individuals are seen to react slower in response to the predator stimulus (Niemelä et al. 2015). These reasons could explain the loss of faster individuals throughout the pre-peak section of their life.

We should be able to measure the boldness of crickets and the average distance they are found from their burrow in the future, using our observational videos. We should also be able to pinpoint the moment of mortality and so discern if these faster crickets were more likely to be predated. This could allow us to distinguish between possible causes of the apparent selective disappearance of faster individuals.

The lack of senescence detected in BS pre-peak could be because reproduction is only just beginning, and the occurrence of selective disappearance of fast individuals could work to maintain slower and possibly shyer individuals in this pre-peak age group (Kirkwood 1977).

4.2 Senescence of mobility in later life

The lack of senescence seen post-peak suggests the quadratic effect of age on the BS of crickets it is driven by the increase pre-peak which merely levels off. After the peak, crickets do not appear to decline in their burst speed, but rather continue to perform at a similar level until their death. The lack of any evidence for selective disappearance post-peak suggests that the negative association of

BS with mortality pre-peak disappears. The lack of any effect on mortality also suggests that BS may not be an indicator of greater intrinsic condition.

It was found that jumping performance was negatively correlated with male mating success in another species of cricket (Lailvaux et al. 2010). If this occurs in our crickets, sexual selection will be biased to slower individuals perhaps causing stronger selection for maintaining condition. This observed maintenance of BS might occur because reproductive success is increased by maintaining physical performance for other reproduction related activities such as singing and searching for mates (Hissmann 1990). It is possible that female BS no longer increases past this point as they have to mature large numbers of eggs, which increases their mass, in turn placing a constraint on their BS, as seen in other cricket species (Ercit et al. 2014). It would be interesting to look at next year's offspring and calculate actual reproductive success; this would allow us to confirm if there is any correlation between BS and reproductive success.

There was no difference in the rate of senescence seen between male and female crickets; this is not as we predicted. When a female cricket is occupying the same burrow as a male, her rate of extrinsic mortality is drastically reduced (Rodríguez-Muñoz et al. 2011). We expected this low level of extrinsic mortality to impact the senescence of females as they do not rely as heavily on BS to survive in later life, hence selection on female BS is reduced and might be expected to show a senescent decline. Possibly males suffering high rates of extrinsic mortality maintain selection on BS and correlated selection prevent senescence in both sexes.

We expected to see selective disappearance of these crickets as they rely on the shelter of their burrows for safety from predators. However, we saw no selective disappearance post-peak. We might have expected to find greater selective elimination in males than in females. This ties in with the previous findings that males have a far higher predation rate after pairing and occupying the same burrow as a female (Rodríguez-Muñoz et al. 2011). However, the interaction of average age and sex was not significant; indicating that there is no selective disappearance post-peak. It seems likely that BS may not be the trait with the greatest effect on risk of predation. It would be beneficial to look at both reproductive efforts and boldness of individuals in the future.

4.3 Variation in burst speed over one escape event

As crickets near the burrow speed slowly decreases (Fig.6). It is apparent from the footage there is first a 'jump' followed by running or walking into the burrow. This pattern of escape has previously been documented in *G. bimaculatus* in response to a stimulus (Hiraguchi and Yamaguchi 2000). The greater the distance from the burrow, the higher the speed reached by *G. campestris* in the initial 10 frames (Fig.4). It has been seen in other species that organisms are able to adjust their escape response depending on how the threat has been perceived; for example deer, lizards, fish and grasshoppers (Amo et al. 2003; Cooper 2006; Stankowich and Coss 2006; Domenici 2010; Domenici and Ruxton 2015). It could be that crickets perceive the distance they are from the burrow and invest more energy to evade the 'predator'. It is also possible that as the cricket is further from the burrow in some cases could be closer to the 'predator' stimulus, potentially causing a greater threat, and greater response. Nevertheless, this is a puzzling observation since one might expect a cricket facing a predator attack to be selected to move as fast as physically possible. The suggestion of strategic allocation to how fast the cricket moves according to its distance from the burrow seems unlikely, but the observed pattern is very clear.

We found a small difference between BS of the sexes, according to their distance from the burrow, with females being seen to move faster than males when further from the burrow (Fig.4). These measures were taken from both pairs of crickets and single occupants. Previous studies of this species have shown that where crickets are paired the male is usually found further from the burrow than the female. It has also been seen that males will preferentially allow females to shelter first (Rodríguez-Muñoz et al. 2011). Perhaps males are slower to allow females into the burrow first; this can only explain the difference when crickets are paired however. It could be that male crickets are slower and so females are simply able to reach the burrow sooner. The speed difference could be down to sensitivity to predator stimulus, females could be more cautious than males when further from the burrow.

Crickets are ectotherms so there is no surprise that there is an effect of temperature on the BS seen. BS is seen to increase with temperature. This is likely due to limitation of BS at lower body temperature as seen in other ectotherms (e.g. Cobb 1994; Cooper Jr 2003). Crickets have a higher metabolic rate at higher temperatures allowing them to run faster.

5. FUTURE PERSPECTIVES

Although studying senescence in the wild involves a number of challenges (Nussey et al. 2008), studies are moving on to learn more about the variation of senescence. This study has shown that a trait that could affect predation likelihood is not seen to senesce *G. campestris* in the wild. We have shown here that it is possible to measure a condition dependent trait that may influence the probability of being predated, in a wild insect over the course of its life.

Due to the nature of the long-term observational video footage collected within the WildCricket project, there are many possible avenues to extend this study further, it will be possible to analyse senescence and BS together with other life history traits. This could include age at first reproductive event and reproductive performance; it will also be possible to look at how many burrows the cricket visited and how far it travelled in its life. It should therefore be possible in the future to look at life history traits in relation to BS and lack of senescence seen here. These possibilities could greatly improve our knowledge of senescence and begin to focus the slightly shadowy picture we currently have.

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CHAPTER 2

Consequences of activity level in a wild insect (*Gryllus campestris*)

ABSTRACT

The variation of lifespan within species is largely determined by the allocation of resources to different life-history traits, and the consequent trade-offs caused by the fact that resources are often limited. The variation of lifespan is a topic that is widely studied, reproductive effort is particularly common; yet despite this we lack understanding of the effects daily activity and performance has on lifespan. We must look at all varieties of trade-offs in order to find how natural selection provides individuals with an optimal life-history strategy. This study set out to see if there were trade-offs seen between various measures of activity and performance on the lifespan of a wild field cricket (*Gryllus campestris*). We also provided food supplements to a number of individuals in the population to assess whether higher quality resources alter lifespan. Using long-term observational data we are able to study these crickets for the majority of their lives, we know the age of almost every cricket, without causing disturbance. Keeping this population totally wild allows us to study lifespan and life-history traits with real environmental effects, such as temperature fluctuations and high predation risk. We found that activity affected the lifespan of crickets; specifically, those crickets that visited a greater number of burrows generally had a shorter life. We were also able to look at male singing effort, and with further data we may be able to confirm that singing at low temperatures reduces lifespan and that the opposite is seen at high temperatures. Speed performance was seen to have no effect on lifespan. By supplementing crickets we were able to see a 29% increase in average lifespan.

1. INTRODUCTION

1.1 Lifespan

It is fair to say that the majority of us strive to live as long as possible, and indeed this is true among most species. However, natural selection works to maximise reproduction, and unexpectedly it seems a long life is not always the answer to this (Kirkwood and Holliday 1979). This is one of the reasons why there are huge differences in longevity among different species, for example, consider the contrast between the lifespan of a human and a field cricket. The explanation for this variation lies in the balance between the benefits of using resources to reproduce as rapidly as possible, and using them to maintain a body that can live for an extended period of time. However, after many years of study the causes of this variation are still only partially understood (De Magalhaes and Costa 2009).

Life-history theory was developed, one aim of which was to explain the considerable variation in lifespan (Stearns 2000). This theory suggests limited resources are shared among life-history traits. These co-adapted traits were described by Stearns (1976) as naturally selected characteristics that allow the organism to adapt to an ecological obstacle. This obstacle could be almost anything preventing reproductive success.

1.2 Variation in lifespan

1.2.1 Variation among species

Lifespan differs strikingly between species. What is remarkable is that even very closely related species, or species that have converged on very similar morphologies often vary greatly in longevity (Austad 2002). Much of the research has included comparative studies to expose the causes of variation in lifespan among species. Large databases of lifespan and life-history trade-offs have enabled extensive comparative analysis, particularly across mammals (De Magalhaes and Costa 2009). These analyses suggest that a major contributing factor to variation in lifespan is variation in risk of mortality, in keeping with evolutionary theories of aging (Medawar 1952; Williams 1957; Kirkwood 1977).

Body size has been well studied and seems to correlate in many cases with longevity, with larger species of mammals tending to live longer (Promislow 1993). A likely explanation for this effect is the decrease in risk of mortality. We also see a similar trend in flying animals (Brunet-Rossinni and Austad 2004). However, over time it has become apparent that size only explains a very small proportion of the variation we see (Promislow 1993). Flight is seen to increase lifespan; this again is suggested to be due to the decreased risk of mortality, seen for example in bats, which have a far longer lifespan than other flightless mammals of similar size (Brunet-Rossinni and Austad 2004). We also see subterranean mammals have increased lifespan, an environment with greatly reduced predation risk (Nevo 1979). However, even with these insights, the causes of variation seen among species remain an interesting and unresolved topic of discussion (De Magalhaes and Costa 2009).

1.2.2 Variation within species

Remarkably, contrary to what we often see among species, smaller individuals within species often out live the larger ones. Larger breeds of dog, for example, age at a faster rate than the smaller breeds, and so die younger (Kraus et al. 2013; Selman et al. 2013). We also frequently see lifespan variation between the sexes, with females often living longer than males (Maklakov and Lummaa 2013). There have been many empirical tests of life-history theory to demonstrate how each individual's life-history is an evolutionary response to maximise reproduction and survival in a given environment (Roff 1992; Stearns 1992; Zera and Harshman 2001).

Selection experiments have shown that increased resistance to stress can also influence longevity. This suggests that some individuals have a genetic advantage over others, as a result of being better adapted to the environment or having fewer deleterious mutations, creating variation in lifespan within the population (Rose et al. 1992). It has also been seen that dietary restriction increases the lifespan of many animals. In the wild there could be huge diversity in food supply within populations which could create lifespan variation (Piper et al. 2005). A recent study demonstrated how condition dependent mortality could increase lifespan through correlational selection; differences in causes of mortality or level of risk among populations could therefore potentially affect the evolution of lifespan (Chen and Maklakov 2012).

1.3 Trade-offs

A huge contributing factor to the variation we see within species is likely to be related to the acquisition and allocation of resources (Van Noordwijk and de Jong 1986). Restricted resources cause a battle between different aspects of life-history, namely investments in reproduction, growth and survival (e.g. (Metcalfe and Monaghan 2003; Biro et al. 2006; Wong and Kölliker 2014)). These clashes of interest are known as trade-offs and have been studied extensively over the years in an attempt to understand variations in lifespan and life-history traits (Stearns 1989).

Part of gaining optimal life-history strategies relies on allocating resources according to what will produce the greatest fitness. Allocation priority changes over the course of life, a juvenile animal would be foolish to spend resources finding a mate when they are as yet unable to reproduce. However, a juvenile putting energy into growing and maturing in early life, then switching allocation to reproduction could clearly increase its fitness (Kaplan and Gangestad 2005).

Energy investments in movement are an often overlooked part of life-history studies (Carbone et al. 2005). Much research into life-history and lifespan evolution focus on trade-offs between reproduction and survival. Level of activity and locomotion performance is a less documented life-history trait, though it seems there are likely to be similar trade-offs with longevity as there are with reproduction (Lailvaux et al. 2010). To understand the costs and benefits of movement it is of great advantage to study animals in the wild. There are many potential causes of the variation seen in the distances moved each day (Irschick and Garland Jr 2001). Most animals must be active to find resources such as food and water, territories and mates (Garland Jr 1983).

1.4 Studying lifespan variation within species

1.4.1 Laboratory vs field studies

Most studies of variation in lifespan have relied heavily on laboratory studies and as such we have a less than perfect understanding of these findings in the natural world (Kawasaki et al. 2008). The lack of a dynamic environment in many laboratory protocols leaves us unable to make accurate and meaningful comparisons between laboratory and field studies. The highly controlled environment of a laboratory is dramatically different to that which we see in the wild. A study of *D. melanogaster* showed that temperature fluctuations can adjust trade-offs, with less investment in reproduction leading to longer lifespans (Marshall and Sinclair 2009). Another laboratory study, in which flies were raised, demonstrated a change in longevity caused merely by enclosure size and even the particular time an investigation is carried out (Kawasaki et al. 2008). When using laboratory studies for empirical information explaining evolution in the natural world, one must take these differences into account.

Studying animals in the wild enables us to look at trade-offs that affect life-history that may either be diminished or not even present in the laboratory. One must take into account factors such as environmental changes which can influence resource availability, and mortality rates which can affect strength of natural selection factors, aspects that cannot simply be replicated in a laboratory (Reznick and Ghalambor 2005). Studies of lifespan variation within species in the wild are few and far between, and many of those that have been undertaken are of mammals. This is likely due to the relative ease of data collection in the wild using capture-mark recapture/re-sight (Marescot et al. 2015). For example, a study of wild voles found trade-offs between reproduction and survival unlike many other studies before, confirming the importance of studying trade-offs with changeable environment and population density (Koivula et al. 2003).

In a study of adaptive variation in senescence of salmon, it was suggested that differing population densities caused by differences in allocation to reproduction and survival caused much of the variation. This pattern is best observed in the wild where there are fluctuations in population size and mortality risk (Hendry et al. 2004). There have been few studies of lifespan variation in the wild, where accurate measures of lifespan can be very difficult. A study on flies (*Telostylinus angusticollis*) found almost no correlation between results from a laboratory and a wild population. It was not possible to see the same differences between sexes, and survival costs were also different between the populations (Kawasaki et al. 2008).

1.4.2 Crickets as a study species for lifespan variation

The field cricket *G. campestris* is an univoltine species and as such it has a single reproductive episode in its life (Masaki and Walker 1987). This species is exposed to high levels of extrinsic mortality (Rodríguez-Muñoz et al. 2011). Due to the great investment in one reproductive event we might expect to see a trade-off between survival and reproduction, with longevity coming at the expense of a lower reproductive rate. *G. campestris* live in the immediate vicinity of their burrows which provide protection from predation (Rodríguez-Muñoz et al. 2011). There is little movement for the first few days before they become mature and start to reproduce (Fisher et al. 2015b). However, after this time crickets are seen to move frequently between burrows in search of mates, suggesting that the likely increase in predation risk associated with leaving the burrow is outweighed by the increased chance of finding a mate (Hissmann 1990).

As ectotherms, temperature has a strong effect on cricket behaviour and activity (Ritz and Köhler 2007). Temperature is also known to influence trade-offs and intra-specific variation in longevity in ectotherms (Marshall and Sinclair 2009; Munch and Salinas 2009). We would therefore expect to see an effect of seasonal temperature on the longevity of *G. campestris*. We would also expect that crickets at higher temperatures may be able to sustain an overall higher level of activity throughout life.

In this study we aim to determine whether there is a relationship between several activity measures and lifespan.

2. METHODOLOGY

2.1 Study species

The field cricket (*Gryllus campestris*) is an Orthopteran found throughout Central and Southern Europe in grassy areas and meadows (Kenyeres 2006; Hochkirch et al. 2008). *G. campestris* overwinter in their burrows as late instar nymphs. The crickets at our study site (in Northern Spain) are known to emerge as adults between late April and May and generally breed between May and July (Fisher et al. 2015a). There are a number of reasons why we have used this species for our study. The fact that individuals stay in very close proximity to their burrows makes it possible to monitor their activity throughout their lives and to determine their time of emergence as adults and death (Rodriguez-Munoz et al. 2010). This capacity to measure behaviour in relation to age and lifespan is essential when looking for trade-offs between reproduction and survival. Basing our study on a natural population allows us to measure lifespan in a population where there is risk of morality by predation. Crickets frequently move from their burrows, often to find a mate, an act which can increase risk of predation (Hissmann 1990).

2.2 Study Site

Data for this study were collected at the “WildCrickets” project field site in Asturias, Northern Spain (Rodriguez-Munoz et al. 2010). The crickets living at this site have been monitored every year from 2006 to 2015 over their breeding season.

2.3 Experimental design and data collection

2.3.1 Monitoring system

Towards the end of April the meadow is scanned daily for active burrows. Each burrow is flagged with an individual number and monitored with a day/night infrared CCTV camera (iCatcher ver. 5.2, www.icode.co.uk/icatcher). Up to 142 cameras are available in a given season. These cameras record video on a computer 24 hours a day at 2 frames per second and allow us to observe the behaviour of individuals over their lifetime. In years where there are more burrows than cameras, the extra burrows are visually inspected, providing a record of the presence and identity of individual crickets.

Using the video recordings and visual monitoring we are able to find the date and time of eclosion from nymph to adult, as well as time of death or disappearance of crickets (Rodriguez-Munoz et al. 2010). Within 2 days of becoming an adult each cricket was trapped, weighed, measured, sexed and tagged with an individual 2 digit code (on the pronotum), for details see: (Fisher et al. 2015a) and (Rodriguez-Munoz et al. 2010). Tagging allows us to follow individual crickets for their whole life, from eclosion as an adult, until they are seen to be predated, no longer emerge from their burrow or found at any other burrow and thus recorded as dead.

2.3.3 Experimental design

To gain a measure of performance and the relationship this has to lifespan, cricket speed was measured in the wild. Throughout the field season of 2015 cricket burst speed (BS) was measured in response to a “predator” stimulus. This was carried out using a remotely controlled slow motion camera set up. The camera recorded at 240 frames per second allowing measurement of the crickets’ BS as they ran into their burrow. Videos were watched frame by frame with Avidemux software (www. avidemux.en.softonic.com). BS of the cricket was measured in number of pixels moved per 10 frames using an onscreen measuring tool ‘small measure’ (www. donationcoder. com/ Software/ Skrommel/ index. html #SmallMeasure). Speed was later converted into millimetres per second (for full details please see methodology in previous chapter).

During the 2015 field season the nymphs in this population were exposed to a 16 day resource manipulation study. This allowed us to look at the effects of increasing nutrient availability on the lifespan of the crickets. Resources were only supplied when all the crickets in the population were nymphs as it is known that they rarely move away from their burrow (Rodriguez-Munoz et al. 2010). Both treatment and control groups were marked by cutting the tip of either right or left hind leg to allow identification in the unlikely event that a cricket moved to a different burrow. Half of the population (treatment) was supplied with fish food pellets *ad libitum*, and the rest (control) were supplied with the same food but covered with a fine mesh so they could smell but not access the food (Holzer et al. 2003).

2.3.2 Data collection

The video footage has been used to create a database of long term behavioural data. Looking for trade-offs between activity levels and lifespan in this study this type of data is invaluable. Each camera covers the area around the monitored burrow where the cricket using that burrow will carry out all their activities. When a cricket leaves the framed area it has left the burrow and moved to a different one. The number of burrows visited by each cricket was used as indicator of activity level, which can be regarded as a mate finding strategy for both male and female crickets (Hissmann 1990).

We were able to use singing effort as a further measure of activity for male crickets. For burrows known to contain a male, we watched 10 minutes of video per hour and recorded whether there was any singing during this time.

2.4 Statistical analysis

All statistical analyses were carried out using R v. 3.2.1. All lifespan data were log transformed for normality unless otherwise stated.

2.4.1 Activity level and lifespan

A number of the explanatory variables in this analysis were lifespan-dependent, such as number of burrows visited and the amount of movement around burrows. In order to convert these variables into lifespan-independent factors we restricted the length of the observation period included in the analysis to 14 days, requiring that the lifespan of all the tested individuals was at least this long. Though losing a number of individuals from our analysis this was a necessary compromise to allow us to examine the effect of phenotypic traits expressed during adult life on adult lifespan. *G. campestris* have an inactive period of approximately 5 days, from eclosion to an adult until sexual activity begins (Fisher et al. 2015b) The observation period included this 5 day inactive period, and the following 9 days with observational data. We selected 14 days as the best range of time, finding the middle ground between including more individuals resulting in a smaller number of observation days or fewer individuals but with more observation days. Using 14 days we were able to include a large number of crickets with enough variation in activity to assess the effects on lifespan.

We calculated burrow rate (BR) by dividing the number of burrows visited by each individual in the 9 day active period by the time it was monitored over that period. As with BR, leaving rate (LR) was calculated using the number of times a cricket left the area around a burrow over the 9 day period, divided by the time that individual was monitored over the period. These two rates ensured there was no bias caused by different times under the cameras during the 9 days. We expect temperature to affect both the behaviour and activity levels of the crickets. Adult degree-day (AD-D) was used as a control for temperature, which is the accumulated time that a cricket was exposed to temperatures within their developmental threshold (10°C) within each season.

We used a general linear model to see if there was an effect of activity level on the lifespan of *G. campestris*. Lifespan was the response variable in this model. Explanatory variables included movement among burrows (BR), activity around the burrow (LR), sex, mass, AD-D and select two way interactions. The model was simplified, using analysis of variance (ANOVA) to compare among models, retaining the simplified model where any reduction in the fit of the model was non-significant at the $p < 0.05$ level (this approach was used for all subsequent GLMs). The final minimum adequate model included; LR, sex and AD-D with interactions between LR and sex, and sex and AD-D.

A separate model was run for singing activity as only males sing. As a measure of singing activity we used singing rate (SR) which was calculated from the number of positive recordings of singing divided by the total number of point samples taken for that particular individual. We used a general linear model to test whether there was an effect of singing effort on the lifespan of male *G. campestris*. Lifespan entered the model as the response variable and singing activity, mass, and AD-D as explanatory variables including all two way interactions.

2.4.2 Burst speed performance and lifespan

As described in the preceding chapter, we took many measures of BS throughout the crickets' lives. However, to avoid any bias, for this analysis we used just the measure from the very first recording per individual, the date of was chosen at random. In the previous chapter, we found an effect of age on BS, therefore this first recording only includes crickets on the left (increasing) side of the curve. To counteract this, we have regressed BS against age and used residual instead of raw speed. We also carried out analysis with maximum speed performance during the first three recordings; however, as the results were so similar we have only included those of the first recording.

A general linear model was used to test the effect of performance on lifespan. Lifespan was not logged as it was normally distributed, this was the response variable. Other factors included in the model were; BS, sex, weight and selected two way interactions.

A general linear model was run to look at the effect of resource supplementation. Lifespan was the response variable and other factors included were: their sex; whether they were treatment or control in the supplementation manipulation; their weight; and all two way interactions.

3. RESULTS

Means are presented with standard errors unless stated otherwise.

3.1 Activity level and lifespan

3.1.1 Movement among burrows

These data were available for 5 years (2007, 2008, 2011, 2012 and 2013). In total 323 crickets were included in our analysis 181 females and 142 males (a mean of 64 crickets each year). Lifespan ranged from 14-72 days (mean lifespan: 32 days), as crickets that lived for less than 14 days were excluded (see methods).

Our analysis showed an effect of the interaction between activity in terms of coming and going from a burrow (measured as LR), and sex on the lifespan of crickets (general linear model (glm): $F_{2,\ 316}$=10.179, p = 0.001). Males that left their burrow more frequently tended to have shorter lifespan whereas the opposite relationship was observed in females. Figure 1 shows that the rate at which individuals moved around burrows in the meadow LR does have an effect on lifespan, and that this in turn is affected by sex.

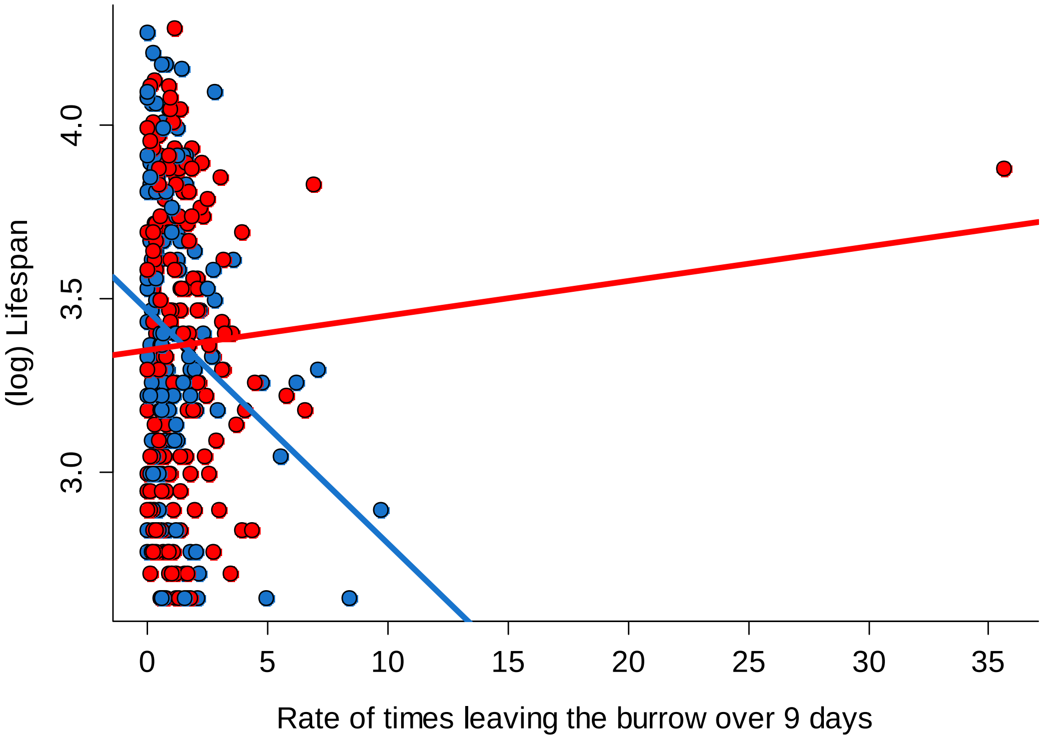

Figure 1. Linear regression of (log) lifespan against LR showing a negative effect for males (blue) and positive for females (red).

As expected we found that AD-D affected lifespan. However, the strength of this effect was dependent on the sex of the cricket (glm: $F_{2,\ 316}$=5.271, p = 0.022). There appears to be an effect of the temperature over breeding season (AD-D), where males tend to live longer than females at higher temperatures (Fig.2).

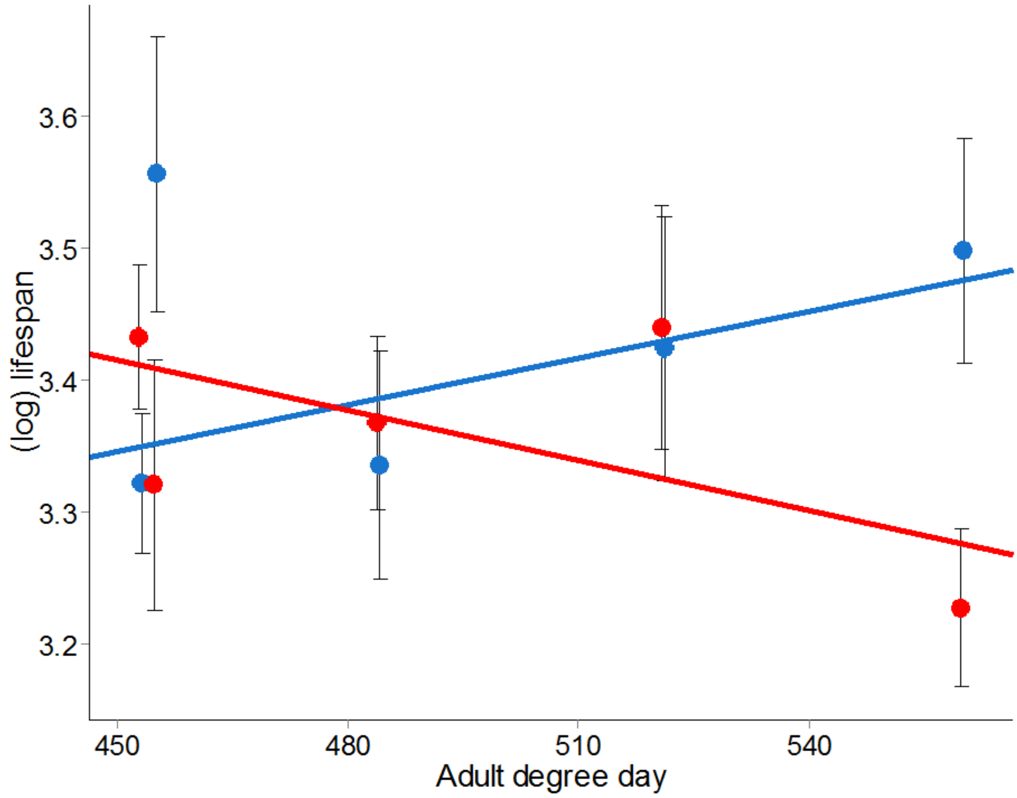

Figure 2. Mean (log) lifespan ± standard error with regression lines showing the effect of season temperature on lifespan, differing between male (blue) and female (red) field crickets.

This analysis revealed a female cricket with an unusually high LR (Female 79, year 2012 from here referred to as F79) (see outlier in Fig.1). For all females including F79 mean LR was 1.46 ± 0.21 and for males it was 1.26 ± 0.13. LR over the 9 measure days for F79 was 35, which is considerably higher than the average when excluding this female (1.26 ± 0.09). To check the reliability of the LR measurement for this female we examined her burrow leaving rate on the days immediately before and after our original observation period. This showed that the 9 day measure period had a LR more than 400% greater than that of the 9 days after this time or the 5 days before, and 30 times larger than the average LR of the rest of the population. There was a period of 4 days (8% of F79's total life) at the start of the 9 day period of which there was an unusually high level of activity around the burrow for F79. Arguably, this suggests atypical behaviour for this cricket for those early days when compared with the rest of its life. The analyses were therefore run again excluding individual F79.

The same method and model were used as before, this time excluding F79 from the data set. LR and the interaction LR x sex were no longer found to be significant (glm: $F_{2,\ 359}$=3.394, p = 0.066), furthermore AD-D and sex were no longer seen to have an effect on lifespan (glm: $F_{2,\ 360}$=3.119, p = 0.078). The final minimum adequate model for this glm included just lifespan and BR. Lifespan of *G. campestris* decreases as burrow rate increases (glm: $F_{2,\ 365}$=6.228, p = 0.013) (Fig.3).

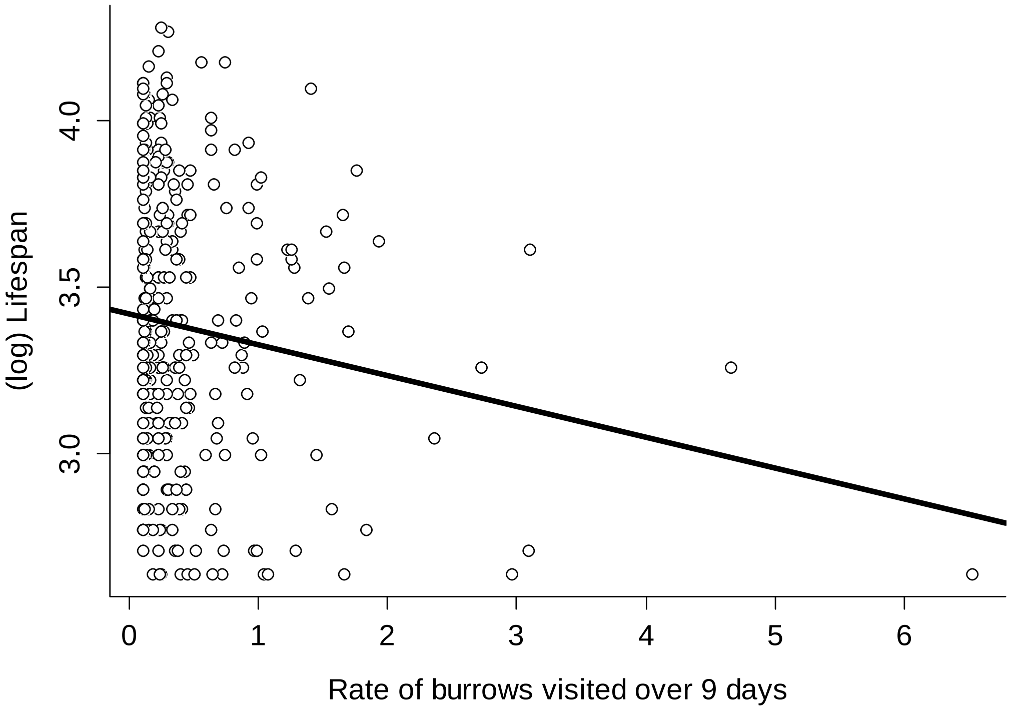

Figure 3. Linear regression of (log) lifespan against BR, showing relationship between movement among burrows and lifespan.

3.1.2 Singing effort

Data for the singing analysis were available for 3 years (2007, 2008 and 2011), including 61 male crickets in total (on average 20 individuals each year) with lifespans ranging from 14-71 days old.

The effect of SR on lifespan was marginally non-significant (glm: $F_{2,\ 57}$=3.737, *p* = 0.058). There seems to be an interaction between AD-D and SR in relation to how it affects lifespan (Fig.4 a-c). In cooler seasons there is a negative effect of SR on lifespan (Fig.4a). Figure 4b shows that at slightly higher temperatures there is a weak negative effect of SR on lifespan. However, as the seasonal temperature rises further it is seen that there is actually an increase in lifespan with higher SR (Fig.4c).

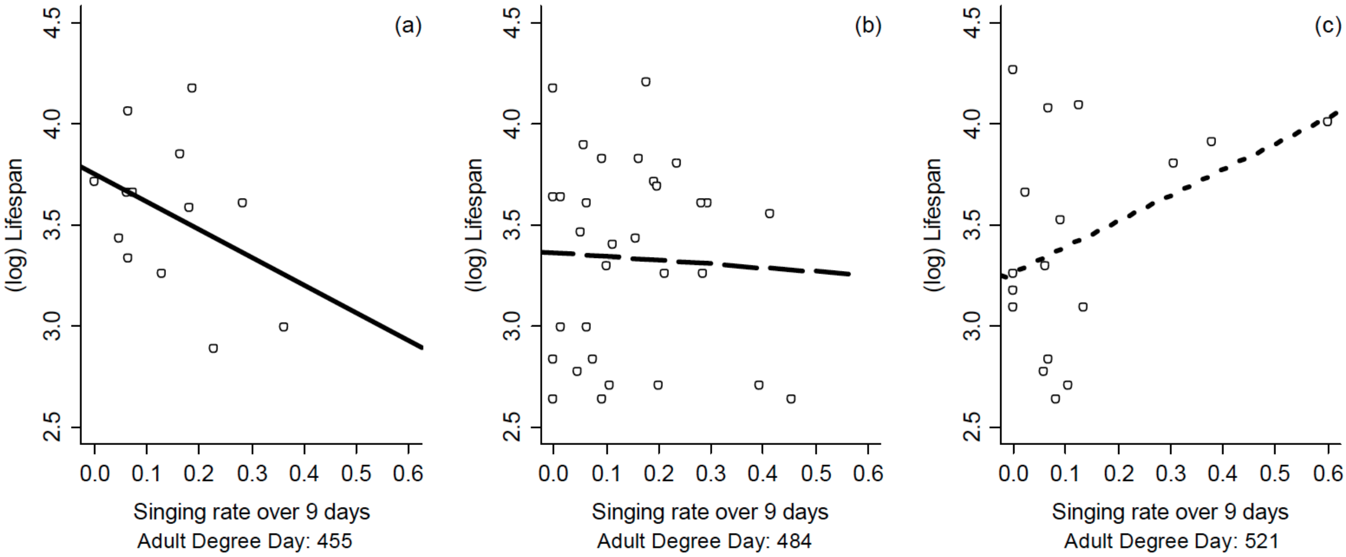

Figure 4. Linear regression of (log) lifespan against SR, varies according to the temperature over a particular season (adult degree day).

3.2 Burst speed performance and lifespan

For our analysis from our 2015 data we had 71 crickets in total (33 females and 38 males). We found no evidence of any potential for BS performance to predict the lifespan of *G. campestris* (glm: $F_{2,\ 69}$=0.882, *p* = 0.175) (Fig.5).

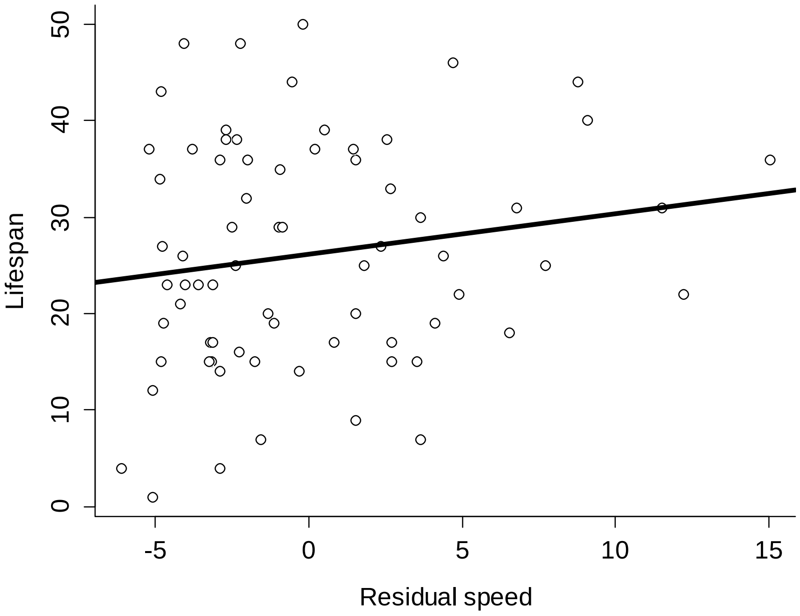

Figure 5. Linear regression of lifespan against residual speed non-significant, *p*=0.175, R^2=0.012

3.3 Resource supplementing and lifespan

In the feeding manipulation 49 crickets were included (29 fed, 20 control). Food supplementation increased individual lifespan (glm: $F_{2,\ 46}$=4.404, p = 0.041). Fed crickets lived on average 29% longer than controls (Fig.6). There were no significant interactions and there was no significant effect of sex (glm: $F_{2,\ 46}$=0.011, p = 0.917) or weight (glm: $F_{2,\ 46}$=1.482, p = 0.229) on the lifespan of these crickets.

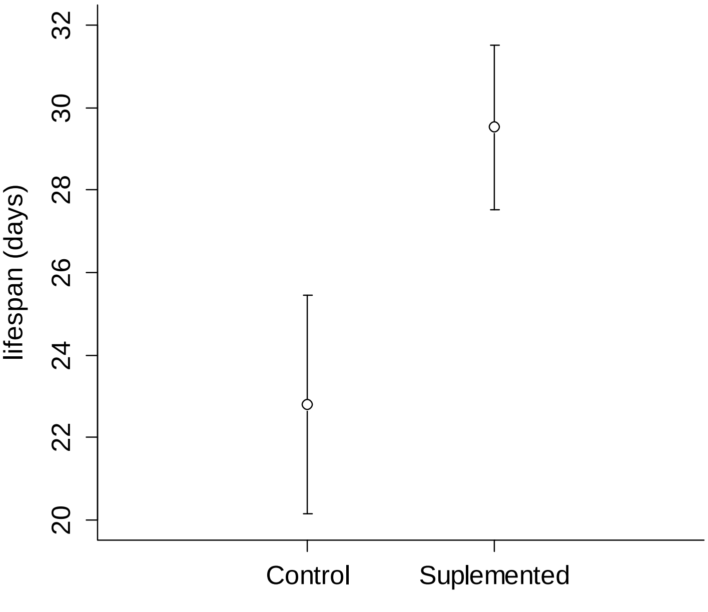

Figure 6. Mean lifespan ± standard error of control group crickets compared to those with a supplemented diet.

4. DISCUSSION

Using our measures of activity level over 6 years and performance levels from 2015, we were able to examine potential a number of traits that trade-off with lifespan. We found that our measures of activity were able to predict some of the variation in longevity in *G. campestris*. We were also able to observe cricket performance in terms of how rapidly they could move, and concluded that this particular measure had no relationship with longevity. Supplementing nymphs with high quality resources increased lifespan considerably.

4.1 Activity level and lifespan

4.1.1 Movement among burrows

Leaves rate

Primary testing on the long-term data of activity around the burrow revealed F79 a female cricket with an unusually high LR. This abnormally high activity level could have been due to F79 using a nearby burrow, moving frequently between the two. It is also possible that F79 was using an area just outside of the camera shot to feed or bask in a sun patch. Further investigations revealed a peak of behaviour at the crickets emergence burrow shortly after the active period started (5 days old), the high LR came from a single burrow. However, F79's behaviour throughout this 9 day period did not reflect the rest of this particular cricket's life (before or after these 9 days), and it was a substantial outlier from the population as a whole. Running the analysis again, excluding F79, made a great difference to the results (both LR and sex interacting with AD-D were no longer significant); thus all further discussion omits the erroneous cricket F79.

Burrow rate

There appears to be a negative relationship between BR and lifespan (Fig.3). It is known that this species use burrows primarily as protection from larger predators and weather (Rodríguez-Muñoz et al. 2011; Niemelä et al. 2015). The pattern we see here could simply be due to a higher chance of predation for crickets that move among burrows; leading to those with a higher burrow rate having a shorter lifespan. It has been seen in wild animals that boldness can reduce survival (Wilson et al. 1993; Réale et al. 2000). Boldness also, however, often increases reproductive success, allowing for this trait to persist (Smith and Blumstein 2008).

It is unlikely that a high propensity to walk is totally separated from reproductive effort. It has been suggested that walking between burrows is an alternative mate finding strategy, and can be an alternative to calling. Generally those crickets seen to be walking will not be calling (Hissmann 1990). In a study by Hissmann (1990) it was found that walking females had a greater number of copulations than those who stayed at the burrow, and males also benefited from walking at high population density. This could mean that BR is correlated with reproductive effort which has been seen to trade-off with lifespan in many animals in many previous studies, e.g. Ground crickets (Fedorka et al. 2004); antler flies (Bonduriansky and Brassil 2002); guppies (Reznick et al. 2004); parasitoid wasps (Ellers et al. 2000).

There are cases where a cricket has to move burrow due to the arrival of another individual. We have frequently seen from video footage that in the event of losing a fight the defeated cricket will always vacate the burrow in search of another. It has been recorded that *G. campestris* of the same sex will rarely share a burrow (Rodríguez-Muñoz et al. 2011). Some high BR measures could be caused by involuntary movement and it is known that those crickets with lower fitness and condition, including smaller individuals are more likely to lose in conflict (Holzer et al. 2003), and may also have shorter lifespans due to their underlying low condition, rather than as a direct result of the fights. This explanation could also explain the reduction in lifespan seen here.

4.1.2 Singing effort

The results showed that singing effort did not have an effect on longevity, significant at the 0.05 level; however with a *p*-value of 0.058, we briefly discuss the possibility of an interaction between singing effort, longevity and AD-D with the intent of collecting more SR data from the years we are currently lacking.

It is conceivable that an increase in temperature could reduce the cost of singing for male crickets. At our lowest recorded AD-D, SR has a negative effect on lifespan (Fig.4a), whereas our highest AD-D has a positive effect (Fig.4c). It is seen in many species that there is a positive correlation between sexual characteristics or displays and individual quality. Many studies have observed that those who invest more in sexual displays live longer as a result of their good condition associated with higher sexual activity (Jennions et al. 2001; Judge et al. 2008). Although we see this pattern emerging at higher temperatures, it is not true at lower temperatures, indeed the opposite is seen.

It is apparent that the effect of SR on longevity changes fairly drastically with increasing AD-D. It has been suggested that investment in sexual displays can have either positive or negative effects depending on the environment (Kokko et al. 2002; Hunt et al. 2004). Our results seem to support the hypothesis of a dynamic relationship between sexual displays and longevity.

Temperature is known to alter cricket call characteristics (e.g. (Martin et al. 2000)) and singing at different temperatures could require different levels of energy (Hoback and Wagner Jr 1997). Perhaps the increased metabolic rates provided by higher temperatures allow more energy for both singing and a longer lifespan. It may be that at lower temperatures crickets must still sing but it is more costly than singing at higher temperatures.

4.2 Burst speed performance and lifespan

Locomotor performance as both a trait requiring maximal effort of multiple systems and a crucial escape mechanism can be an indicator of condition and longevity in crickets. (Lailvaux and Irschick 2006; Lailvaux et al. 2010). In contrast to our prediction we were unable to see a trade-off between burst speed and lifespan in these field crickets. This suggests that although high BS is not something that can only be achieved by crickets in sufficiently good condition and can live a long time, expending greater energy in this antipredator behaviour certainly does not shorten lifespan (Fig.5).

As BS is known to be an investment of maximal effort to escape a predator, it could be that crickets that allocate a large amount of energy to this throughout life, decrease their limited energy resources faster than others. Therefore, although they are able to avoid predation they might senesce sooner than a cricket who invests less in BS, thus seeing no effect on lifespan. It may be that other factors and natural extrinsic mortality could mask any trade-offs, and it would therefore be beneficial to collect more BS data over a number of seasons to increase the power of our analyses in the future.

4.3 Resource supplementation and lifespan

We found a large increase (29%) in lifespan as a result of supplementing *G. campestris* nymphs with a rich food source. This reinforces the prediction that there is a limitation in the amount of energy crickets can extract from their natural food (grass), and that an increase in energy reserves allows crickets to live longer. This has been seen in many previous studies (Jennions et al. 2001). It is possible that this difference in longevity is caused by reduced investment in risky foraging behaviours, however, as all individuals were supplemented before adulthood this seems unlikely. It is improbable that this huge difference seen is due to heightened predation for those who were not supplemented.

The pattern we have seen here through dietary supplementation in the wild is reminiscent of dietary restriction studies carried out in the laboratory. Dietary restriction in the laboratory is seen to increase lifespan; even recently derived wild populations (of *Drosophila*) have shown this pattern (Metaxakis and Partridge 2013). Most studies of restriction provide limited levels of carbohydrate and protein without causing starvation. In the laboratory restriction experiments it is seen that although lifespan increases for both sexes there is often reduced reproductive success for females (e.g. *Teleogryllus commodus* (Maklakov et al. 2008)).

There was no difference seen between male and female crickets, as has previously been seen in resource supplementation experiments in the laboratory (Hunt et al. 2004). Our results suggest that neither male nor female crickets in our wild population allocated resources into reproductive effort at the expense of longevity. However as our results are reminiscent of those seen in dietary restriction studies it would be interesting in the future to look at the investment into reproduction of supplemented crickets to deduce if the same is seen when supplementing crickets in the wild.

Currently we are unable to look at these effects of resource supplementing on other aspects of *G. campestris* life-history traits. When the observational videos of 2015 have been analysed, we will have numerous characteristics to look at. This could help to explain this great increase in longevity. It would be particularly interesting to look at singing rate in males and reproductive output, allowing us to look for trade-offs. It may even be possible in the future to look at the offspring of these supplemented crickets and look at the impact on their actual fitness.

5. FUTURE PERSPECTIVES

This study has given us an insight into the variation seen in lifespan of a population of wild crickets. In the future it will be possible to link these findings with more observational data on individual behaviour and life-histories. This may enable us to link life-history traits such as the onset of reproduction with behavioural traits such as movement and maximum escape speed. Due to the nature of our study and our potential to establish a pedigree, we may be able to examine lifespan variation among siblings, helping to disentangle the effects of genetics and environment.

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CHAPTER 3

Concluding Chapter

1. INTRODUCTION

The study set out to look at senescence and lifespan variation in a wild population of field crickets (*Gryllus campestris*). We were able to identify patterns of senescence and disentangle the effects of selective disappearance looking at senescence both between and within individuals in the population. The study also investigated activity levels and performance and sought possible trade-offs between these and longevity, as well as the effect of a supplemented diet on lifespan. Current literature is dominated by laboratory studies, but the importance of wild studies is becoming recognised to a greater extent. Our study aimed to answer the following questions:

- Is there senescence seen in a trait directly linked with predation risk?
- Are there trade-offs between lifetime patterns of activity including reproductive traits and lifespan?

2. EMPIRICAL FINDINGS

The outcomes of this research are summarised below. A detailed description can be found within the preceding chapters:

1. Senescence of a predation related trait in a wild orthopteran (*Gryllus campestris*)
2. Life limiting consequences of an active lifestyle in a wild insect (*Gryllus campestris*).

In this chapter we combine all our findings, seeking to answer the main questions of this study, and how these answers can contribute to current knowledge.

Question:	Findings:	How does this contribute to current knowledge?
Is there any evidence of senescence in a trait linked with predation risk?	No.	Our evidence fails to support the current body of research confirming that senescence is widespread in natural populations (Nussey et al. 2013). Our findings suggest that sexual selection could maintain BS over later life. Individuals able to maintain reproductive performance can also maintain BS (Lailvaux et al. 2010). We have begun to fill the gap in information on patterns of senescence in populations with differential predation rates.
Is there evidence for non-random mortality?	Yes, selective disappearance of faster individuals was seen in early life.	Our research confirms the importance of disentangling the effects of non-random mortality from within individual senescent changes (van de Pol and Wright 2009). We saw an increase in BS over the first 12 days of adult life which was not reflected in the population as a whole, which is presumably explained by selective disappearance of fast individuals. This is the opposite direction of the findings of other natural studies with high extrinsic mortality (Hämäläinen et al. 2014). It remains vital to look at within and between individual differences in the study of senescence (van de Pol and Verhulst 2006).

Question:	Findings:	How does this contribute to current knowledge?
Is speed performance in fleeing crickets altered by temperature?	Yes, there is a positive relationship between speed and temperature.	This finding agrees with the literature about the fact that ectotherms are able to move faster at higher temperatures. It is therefore important to account for temperature to avoid bias when looking at any movement related trait such as speed in the natural environment, where temperature is a variable factor (Lehmann 1999; Norris and Kunz 2012).
Is there a sex difference in fleeing speed?	Yes, females are faster than males both at higher temperatures and over longer distances.	A sex biased mortality rate has previously been seen in *G. campestris*, where paired males are at a greater risk of predation than females (Rodríguez-Muñoz et al. 2011). Our findings tie in with this, when there are two crickets at the burrow the female will reach cover first, leaving the male exposed and liable to predation.
Is there a relationship between speed performance in fleeing crickets and lifespan?	No.	Escape performance as a maximal expenditure of energy can be a display of condition and longevity in crickets (Lailvaux et al. 2010). However, we failed to find such a relationship, suggesting performance may not always be an indicator of quality, or affect lifespan.

Question:	Findings:	How does this contribute to current knowledge?
Is there a trade-off between number of burrows visited over life and lifespan?	Yes, there is a negative relationship between number of burrows visited and lifespan.	Assuming that visiting burrows increases reproductive success, by increasing chances of finding mates, our result is at odds with the literature suggesting greater investment in reproduction is associated with longer lifespan (Jennions et al. 2001). Our results however, are consistent with the finding that higher quality individuals may die younger as a response to high investments in reproductive activities (Hunt et al. 2004).
Is there a trade-off between singing activity and lifespan?	No, though an interaction of singing effort and seasonal temperature results were extremely close to significance.	The literature suggests a clear relationship between reproductive effort and lifespan (either positive or negative), but this might not be so simple (Jennions et al. 2001). The difference in results from year to year suggest there may be an important influence of environmental conditions on life-history trade-offs. These results support the concept of a dynamic relationship caused by variable environmental conditions (Kokko et al. 2002; Hunt et al. 2004).

Question:	Findings:	How does this contribute to current knowledge?
Is longevity affected by resource acquisition?	Yes, rich food supplementation caused a 29% increase in lifespan.	This result coincides with many other studies in finding that an increase in the amount of energy stored increases lifespan (Holzer et al. 2003; Hunt et al. 2004). Although studies have also found differences between the sexes, with males living shorter than females, we did not find any indication of this (Hunt et al. 2004).

3. LIMITATIONS

The study has offered empirical data on senescence and lifespan variation, in a wild population of *G. campestris*. As a direct result of our methodology there are a number of limitations on the questions that we can address and the answers that we can provide.

3.1 Equipment

3.1.1 Data collection issues

The slow motion camera equipment required for recording burst speed data had a number of minor issues which together meant that recording was not 100% reliable. This included the longevity of the camera batteries and the consistency of the remote triggers. Camera battery life lasted approximately 4 hours; with an hour break to recharge this often gave enough power to complete recording each day. However on some ‘heavy’ recording days there was simply not enough battery to take full advantage of all the time with high cricket activity. The triggers were fixed to the top of the camera however as the camera became warm in the sun the fixings became more flexible. In these cases there was occasionally too little resistance for the trigger to depress the record button. These issues caused a loss of data which would have otherwise been beneficial to the project. The more recent model of the same camera now has an inbuilt Wi-Fi remote trigger which will greatly assist future attempts to conduct a similar study.

3.1.2 Altered behaviour

The camera trigger system we used caused a small amount of movement and noise. The vibrations travelling down a nearby grass blade occasionally inadvertently initiated a flight response from the cricket into the burrow. This meant that a number of the recordings only caught the end of a burst speed event, which could not therefore be used. This would have resulted in fewer recordings for a cricket and therefore less data. It is also possible that the greater number of fleeing responses resulting from our stimuli could have altered the crickets’ behaviour on days when they were tested, although escape responses are a common behaviour, so it is unlikely this would have been a major issue.

3.2 Problems related to working with a wild population

3.2.1 Diet supplementation

Using our observational system many of the usual problems associated with natural studies can be overcome. However the diet supplementation manipulation caused a significant loss of data points from the measurable population. This was the result of us losing track of those crickets that were fed and those in the control group. Although the nymphs had a leg clipped according to the treatment group, once a nymph had eclosed into adulthood it was not always possible to match up the two especially if it had moved from the original burrow. This significantly reduced the sample size for this study; although we had adequate numbers a greater number could have improved statistical power.

3.2.2 Weather

There was constant high rain fall on a number of days; this left us unable to do any burst speed analysis as the camera trigger equipment was not waterproof. In total we lost a total of 3 days recording which if included could have given up to 150 more burst speed videos in total. Two of the 3 rainy days were consecutive, this caused there to be a slight gap in many of the recordings that were otherwise taken throughout life.

3.2.3 Seasonal changes

There were undoubtedly unavoidable seasonal changes throughout the data collection of this study. As the season progressed the temperature increased, as does the age of *G. campestris*. There is therefore a covariance between age and season, meaning that older crickets could have been recorded at higher temperatures. However temperature was included in all analyses to counter this variation over season, this should drastically reduce the likelihood of any problems caused by seasonal change.

4. FUTURE RESEARCH

Even at the local population level, there is high variability in longevity in *G. campestris*. Interestingly, we observed no senescence in a trait linked to predation. To develop our understanding of senescence with regards to evolution in the wild, it seems there is a need to measure the strength of both natural selection and sexual selection on the traits of interest. We were unable to incorporate actual reproductive success into this study, however in the future this will be essential to understanding how variation in lifespan and senescence came about in the wild (Archer and Hunt 2015). There are a number of ways this incorporation of sexual selection and reproductive effort could add to our understanding of the evolution of senescence in the wild.

When the observational video from 2015 has been analysed we should be able to ask some interesting questions regarding speed performance and reproduction. It will be particularly interesting to analyse whether reproductive performance is related to the maintenance of speed. As the crickets increase in speed up to a point it would be interesting to see if the point at which speed ceases to increase is associated with either onset of reproduction or perhaps weight change over the season. We were only able to use a single weight measure to cover the season in most cases. Collecting weight data throughout the season should enable us to determine any effects that egg load may have on speed performance. This has been seen to both slow maximum speed and likelihood of predation, but would only account for the effect seen in females (Cooper Jr et al. 1990; Ercit et al. 2014). By looking into this further we may be able to confirm the reasons for maintenance of BS that seem to suggest that there is no senescence of BS in this population.

It would be interesting to analyse changes in singing rate over the season to see if this follows the same pattern found in speed performance, where males do not show a decline with age. This will make it possible to test whether there is a positive correlation between burst speed and singing effort, and between burst speed and the number of burrows visited. We hypothesise that burst speed follows a similar pattern to both reproductive traits (walking between burrows and singing (Hissmann 1990)). If there is a genetic correlation between these traits, sexual selection could be exerting correlational selection for faster crickets. We could, therefore, be able to confirm that although natural selection is weakening over individual lifespans sexual selection keeps this trait at high performance throughout later life. Looking at the effects of sexual selection on senescence could prove useful when deciphering the causes of variation seen in the wild (Archer and Hunt 2015).

It would also be beneficial to collect further speed performance data over next years to allow us to look at effects on predation rate through further seasons. Our DNA profiling will allow us to compare lifespan and senescence in siblings and also between parent and offspring. This will then allow the partition of variation between genetic and the environmental factors, perhaps enabling us to see if speed performance in relation to avoiding predation is heritable. In future we should also be able to see how lifespan is affected by the environment by comparing closely related individuals.

Although we observed selective disappearance of faster individuals in early life, we were unable to see an effect of cricket speed performance on their lifespan. It would be beneficial to look at the causes of this selective disappearance in early life and why it seems to disappear in later life. Using our observational footage from 2015 we will be able to identify the cause of death for many of the crickets. This should allow us to determine whether mortality is truly linked directly to speed (for instance predation) or whether speed and mortality are both correlated to a third factor, such as boldness.

To gain further knowledge of the trade-offs experienced in this population we could include onset of reproduction in analysis of lifespan variation. Adding to the already growing body of literature, this information could help to give an insight to the variation in senescence and longevity (Roff 1992; Stearns 1992). It would be interesting to add further years of singing data to the lifespan variation investigation as this could help to find if there truly is a pattern between seasonal temperature and how singing affects longevity. It would be interesting to look at the average age at start of reproduction over different seasons. This could enable us to see if the difference in lifespan could be caused by temperatures adjusting the onset of reproduction and consequently the lifespan of the population as well.

5. CONCLUSION

This research contributes valuable information to the field of biogerontology, giving original insights into a wild population of a relatively short lived orthopteran. By monitoring the population over many years and collecting detailed field data we provide evidence that we cannot see senescence in the BS of this population. It has also become clear that there are links between activity levels and lifespan though further investigations are needed to draw stronger conclusions. We also found exciting evidence for lifespan lengthening effects of resource supplementation which will provide the potential for further study questions in the future. We have shown how such a long-term and thoroughly observed population such as this can be advantageous to the study of senescence and lifespan variation.

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