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Abstract

Courtship and mating are costly and as a result, organisms are faced with trade-off decisions between the costs and benefits which arise from them. Individuals use information on their own quality and the attractiveness of their potential mate to determine whether to court and mate, and if so how much energy to invest in these activities. An individual's quality can affect its ability to perform sexual activities and determine the energy the individual is able to invest in such activities. Organisms assess one another on their relative attractiveness, which allows them to determine the relative benefits they will receive from courting and mating with them. How quality and attractiveness affects the decisions which organisms face when presented with a courtship or mating opportunity is not fully known. This study altered the quality and attractiveness of *Drosophila melanogaster*, through the manipulation of nutrient levels in their diets, to investigate how this would affect the decisions and energy investments that they made towards courtship and mating behaviours. Results showed that courtship latency was unaffected by differing levels of quality and attractiveness, while courtship frequency was affected. No results were provided for how mating behaviours were affected by quality and attractiveness. This study concludes that an organisms level of attractiveness is the combination of various traits which can each have different influences on courtship behaviour, leading to different trade-off decisions being faced. An individual's attractiveness is also relative to the quality of their potential mate.

Introduction

The complex manner in which organisms decide when and who to court and mate with is influenced by both their own quality, and the attractiveness of their potential mate (Holveck & Riebel, 2009). This decision that organisms must make can be looked at in the context of trade-offs as energy spent on reproduction can reduce survival and future reproductive success (Fowler & Partridge, 1989), with the effort invested in courtship (Simmons et al., 1992) and mating (Fowler & Partridge, 1989) being costly factors in that decision. The potential costs of courting and mating in this trade off decision can be influenced by the amount of energy an organism has available, which is generally linked to the quality of the individual (Lerch et al., 2011), as with increased quality comes increased energy available for these activities (Schultzhaus et al., 2017).

In addition to these trade-off decisions, the attractiveness of their potential mate also plays a role in the decision of whether to court or mate. Theories of sexual selection suggest that the most attractive individuals will successfully mate the most and produce the most offspring (Andersson & Simmons, 2006), therefore achieving the ultimate goal of increasing their fitness (Cornwell & Perrett, 2008). An attractive individual is one that possesses the traits which the opposite sex has a preference for in order to achieve increased fitness though good quality offspring (Bos et al., 2009). If the decision on whether to court and mate is considered from a trade-off perspective alone, then a low-quality individual may not be expected to expend their limited energy on courting and mating if the costs are too high. But when the attractiveness of the potential mate is accounted for in this decision, it may change this, as if the attractiveness of the mate is high enough, then the costs of courting or mating could be outweighed by the benefit from the potential fitness gained from reproducing (Schultzhaus et al., 2017).

A potential way of answering the questions brought about by these decisions that organisms make is to manipulate their own quality and the attractiveness of their potential mate. A mechanism for this manipulation is to alter their diet and its nutritional levels which in turn alters their quality and condition through affecting the animal's energy levels, with individuals on higher quality diets having higher energy reserves (Skorupa, et al., 2008). In many insects it has been found that altering their quality by the manipulation of nutrient intake can have marked effects on the male's ability to successfully attract females and achieve mating (Shelly, et al., 2002). It is thought that there are two major components of the individual's reproductive behaviour that a reduction in quality can have impacts upon. Firstly being their sexual activities, which include their performance of courtship signals (Landolt & Sivinski, 1992; Droney, 1996), and secondly their investment in synthesising the products needed or transferred during reproduction, including pheromones (Löfstedt, 1989) and sperm (Pitnick & Markow, 1994).

The way in which an individual's quality and attractiveness can affect its sexual activities has been documented in many insect species, although many of these predominately focus on the male sexual activities rather than that of the female. The quality of bush crickets, *Requena verticalis*, manipulated by diet quality, was found to have an effect on the frequency of mating attempts, with low-quality individuals performing a reduced number of mating attempts compared to the number seen from high-quality individuals (Schatral, 1993). The study also found that low-quality males

rejected mating attempts from females more often than their own mating attempts were rejected by the females. This may be the result of a trade-off that the low-quality males have been faced with as the cost of reproduction could be too high in comparison to the benefits available. Gwynne (1993) found that a reduction in quality of Mormon crickets, *Anabrus simplex*, resulted in reduction in the number of sexually active males and a showed reversal of the normal courtship and mating roles with males being the discriminating sex. At the extreme, some studies found that a reduction of quality and attractiveness, through diet manipulation, can partially or completely interrupt courtship and mating in insects (Adam & Nelson, 1990; Foster, 1976; Bartell et al., 1969).

It is evident from studies that a change of quality and attractiveness has an effect on the decisions that insects have to make towards courtship and mating in some way. This study set out to investigate how the relationship between an individual's own quality and the attractiveness of a potential mate affects the decisions which they make towards courtship and mating, using *Drosophila melanogaster* as a model for this. The use of *D. melanogaster* in studies concerning these decisions is very common (Holveck & Riebel, 2009; Manning, 1967; Ryner et al., 1996). They are a useful animal model to use as it is known that courtship and mating are costly to them (Cordts & Partridge, 1996; Fowler & Partridge, 1989). It is also understood that their quality and attractiveness can be manipulated through the altering of their diets (Janicke et al., 2015).

Male *D. melanogaster* will court females by performing an elaborate and innate courtship ritual (Demir & Dickson, 2005) while females will not court the males. This courtship ritual can be broken down into three main aspects; orientation, vibration and licking, which are then followed by the male mounting the female, providing she is sufficiently aroused and accepts his courtship advances by opening her vaginal plates (Demir & Dickson, 2005). The orientation involves the male orientating himself towards the female, this orientation is maintained throughout the courtship of the female with the other stages built upon this (Bastock & Manning, 1955). The vibration stage joins the orientation act, this is characterised by the vibration of the males extended wing, which creates a species-specific courtship song which is thought to allow the females to recognise con-specific mates (Ewing & Bennet-Clark, 1968). Finally comes the licking stage, this involves the licking of the female genitalia by the male, with often numerous attempts at this before copulation is accepted by the female (Manoli & Bake, 2004). If the males' advances are accepted by the female then copulation proceeds by the male curling his abdomen around the female during mounting (Ryner et al., 1996). If his advances are rejected by the female, he will either restart the courtship ritual in another attempt to arouse her or stop courting her (Bastock & Manning, 1955).

While the female is often described as the 'passive' sex during these courtship rituals, they do in fact show some rejection responses which can have deterrent consequences upon the courtship from the male (Cook & Cook, 1975). These rejection responses can be brought about for a number of reasons, including the female's stage of sexual development, if she has already recently been inseminated, or if the courtship from the male is inadequate (Manning, 1967). This suggests that while males take a more active role in the courtship, the females have an influence in the duration of courtship by the amount of fleeing they carry out (Partridge et al., 1987), and whether courtship results in mating, through rejection or acceptance of courtship.

In field populations, *D. melanogaster* consume rotten fruit which provides them with a diet composed mostly of sucrose and protein, from the yeast which colonises these fruits (Schultzhaus et al., 2017). To simulate this in laboratory environments, sucrose and yeast are common ingredients used in the culture media provided to *D. melanogaster* (McRobert, 1986). Sucrose and yeast were therefore the two substances manipulated in the diet of *D. melanogaster* in this study. The objective of this study was to find out how differing levels of an individual's own quality and the attractiveness of a potential mate, manipulated by altered levels of sucrose and yeast in their diet, affected decisions and effort invested towards courtship and mating by both males and females, using *D. melanogaster* as a model for this. This was achieved by recording the males' latency and frequency of courtship attempts and the frequency of mating between males and females in pairings made up of individuals of different quality and attractiveness and of similar quality and attractiveness. The results from this aimed to give further understanding around the tactical decisions that males and females must make, not only when choosing when and who to court and mate with but also in decisions involving the potential increase of their own fitness.

Methods

Fly husbandry and collection
This study used laboratory strain Oregon-R (OR) *D. melanogaster* stored in vials (23mm in diameter, 95mm in height) for multiple generations, incubated at 26°C with a 12:12 h light:dark cycle. Flies had been maintained on the laboratory's standard diet (*Drosophila* agar, 14g/litre; yeast, 40g/litre; sucrose, 40g/litre; made up to 1 litre with distilled H_2O; with methyl-4-hydroxybenzoate, 40ml/litre added after heating and then cooling below 50°C). Flies were separated into two groups, one being kept on the standard laboratory diet (high nutrient), with the other changed to a low nutrient diet (*Drosophila* agar, 14g/litre; yeast, 20g/litre; sucrose, 20g/litre; made up to 1 litre with distilled H_2O; with methyl-4-hydroxybenzoate, 40ml/litre added after heating and then cooling below 50°C). These two diets of differing nutritional levels allowed for the quality and attractiveness of the flies to be manipulated to high-quality and attractiveness (reared on the high nutrient diet), and low-quality and attractiveness (reared on the low nutrient diet).

Following 14 days of incubation to allow time for flies to mate, lay eggs and for eggs to develop through to final pupal stage, adult flies were removed from vials, leaving only pupae. This was done between 09:00 and 11:00 GMT, after which the vials were returned to the incubator until 16:00 GMT. Any flies that were found in the vials following this period of incubation were virgins, as would have emerged from pupae earlier in the day, therefore having no interaction with mature flies. These were then anaesthetised by putting the vials in ice and their sex was determined by eye with the aid of a laboratory microscope. Male and female flies were separated into single sex vials with the appropriate diet (high or low) provided. Virgins were then incubated at 26°C with a 12:12 h light:dark cycle for 48-72 hours.

Experimental procedure - mating assay
Four combinations of pairs of flies were recorded in a mating chamber in this study, with 12 pairs making up each group, (1) high-quality males with high-quality females, (2) low-quality males with low-quality females, (3) high-quality males with low-quality females, and (4) low-quality males with high-quality females. The mating chamber

(shown in Figure 1) was similar to the 'Copulatron' created by Drapeau & Long (2000). It consisted of 16 circular holes (10mm in diameter and 7mm in depth), with two removable clear plastic sheets. Sheet A covered the top of the chamber's holes and secured the flies. Sheet B allowed for male and female flies to be kept separate. Female virgin flies were placed in separate holes in the chamber, after being anaesthetised by putting vials in ice. Sheet B was then inserted into the chamber to secure the flies in the holes. Anaesthetised male virgin flies were then each placed in separate holes above sheet B, and sheet A was then inserted above them. The mating chamber was then placed in an incubator set to 26°C and left for 10 minutes prior to recording to allow for flies to acclimatize. After this, a recording of the chamber was started using a high definition webcam (Logitech HD c920) and sheet B was removed so that male and female flies were no longer separated. Each recording lasted 60 minutes from the time of sheet B being removed.
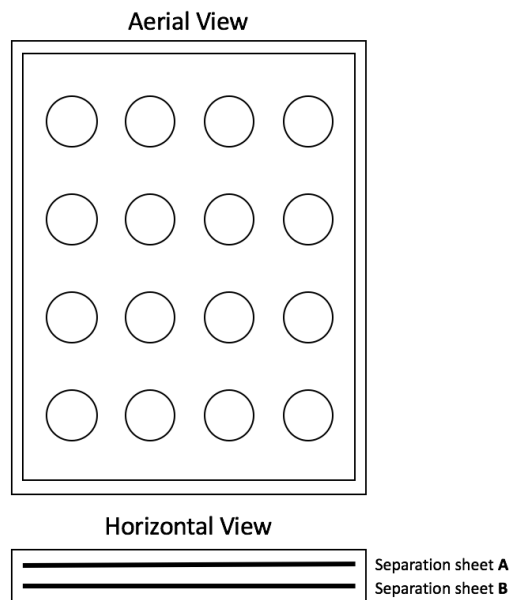
Aerial View

Horizontal View

Separation sheet **A**
Separation sheet **B**

Figure 1: Diagram of mating chamber, showing aerial view and horizontal view. The aerial view shows the 16 circular holes in the chamber. The horizontal view shows where the two removable separation sheets are inserted. Sheet A is at the top of chamber covering the tops of the holes, sheet B is below sheet A allowing for flies to be kept separate.

Analysis of courtship and mating behaviour
As the focus of this study was on the courtship and mating decisions that males and females made, the behaviours that were measured related to this. To determine investment in courtship, the male's courtship behaviours were recorded, and to determine investment in mating, the mating behaviours were recorded. Courtship Latency (CL) (the time between the removal of sheet B and first attempt at courtship from the male), Courtship Frequency (CF) (the number of courtship attempts in the five minutes following the first courtship attempt from the male) and Mating Occurrence (M) (the time until mating occurred and duration of mating) were measured. Courtship attempts measured in this study was defined as the act of the male directing a 90° wing extension and vibration towards the female (Turiegano et al., 2013). Every time

the male extended his wing in this way towards the female and then returned it to its normal position, it was counted as a single courtship attempt.

Statistical procedures
Statistical analysis was carried out using IBM SPSS Statistics for Macintosh, Version 23.0. Correlation between CL and CF was first tested using a non-parametric correlation test, as count data was non-normal, to find the Spearman's rank-order correlation. This test was then repeated, but this time comparing the sub-groups of quality levels. This allowed correlation between CL and CF across the different combinations of quality levels to be tested. To test whether the quality and attractiveness of females and males had an effect on CL and CF, two Univariate Analysis of Variance (ANOVA) tests were carried out for both CL and CF, using the logged data for each, with each set of residuals conforming to normality.

Ethical note
The protocol used in this study was approved by the Plymouth University, School of Biological and Marine Sciences ethics and welfare review process. *D. melanogaster* were used in the study as they are not protected under the Animals (Scientific Procedures) Act 1986 (Home Office, UK, 1986), and therefore follow the NC3R's 'replacement' guideline. Following the NC3R's 'refinement' to minimise damage and discomfort to the flies used in the study, flies were euthanized by submersion in ethanol and freezing after use. Difficulties arose when trying to fulfil the NC3R's 'reduction' guideline as large numbers of flies were needed in order to maintain the population of flies for use in the study, although efforts were made to not be wasteful in the number of flies used (NC3R's, 2017).

Results

Mating behaviour
Only three out of the 48 pairs of flies in the study mated. As a result of this, no statistical tests were performed on this particular measurement and this aspect of the study was disregarded from further results and analysis.

Is there a relationship between courtship latency and courtship frequency?
Courtship latency and courtship frequency may both be measures of male motivation to mate. There was no significant relationship found between CL and CF (Spearman's rank correlation: r_S = -0.196, N = 46, P = 0.191). The time in which it took for a male to begin courting a female did not have an influence on the subsequent number of courtship attempts that he made. There was no significant relationship between CL and CF across each of the levels of quality and attractiveness (Spearman's rank correlation: r_S < -0.189, N = 10-12, P > 0.144). This shows that there is no relationship between CL and CF regardless of the quality and attractiveness of the males and females.

Does quality and attractiveness affect courtship latency?
There was no interaction between male and female quality and attractiveness on CL ($F_{1, 42}$ = 2.504, P = 0.121), meaning that the effect of quality on females did not depend on the quality level of males, and vice versa. Female attractiveness showed no effect on the CL shown by the males ($F_{1, 43}$ = 2.075, P = 0.157), meaning that males were equal in their latency to court relatively attractive and unattractive females

(see Figure 2). Male quality also showed no effect on their CL towards females ($F_{1, 43}$ = 0.131, P = 0.719), meaning that males of high and low-quality began courting females within equal times (see Figure 2). This suggests that quality and attractiveness of both males and females does not play a role in determining the latency of courtship.
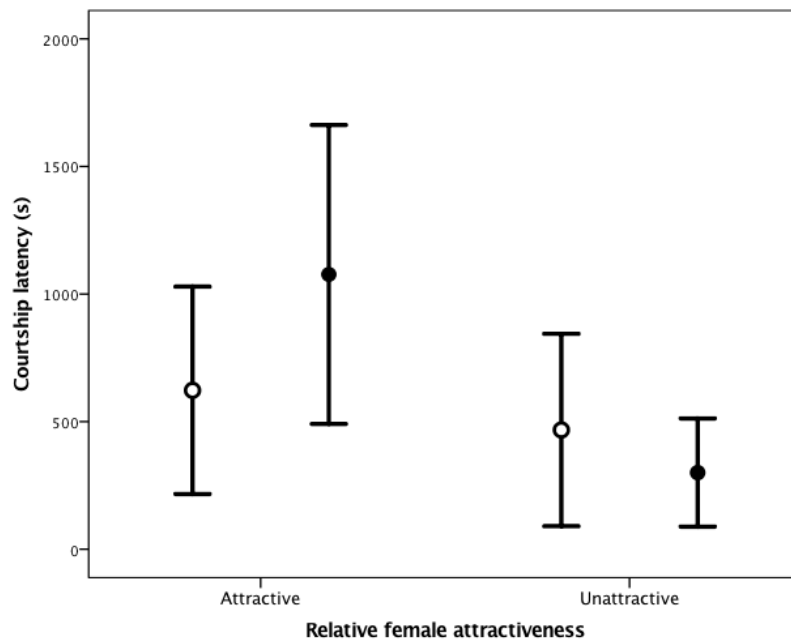

Figure 2: Courtship latency (y-axis) in relation to sex and quality and attractiveness showing that both relative female attractiveness (x-axis) and male quality (high-quality: open circles, low-quality: closed circles) did not have a significant effect on courtship latency. Attractive and unattractive females did not receive significantly different latency in courtship attempts, from either quality level of males. The error bars show the 95% confidence interval of the mean. Raw data was used in this figure, rather than the transformed data used in the statistical tests.

Does the nutritional level of males and females affect courtship frequency?
There was no interaction between male and female quality and attractiveness on CF ($F_{1, 44}$ = 0.126, P = 0.724), meaning that the effect of quality on females did not depend on the quality level of males, and vice versa. Female attractiveness influenced the number of courtship advances they received from a male ($F_{1, 45}$ = 7.678, P = 0.008). Relatively attractive females received more courtship attempts from males than unattractive females (see Figure 3). Similarly, a male's quality had an effect on the number of courtship advances that he made towards a female ($F_{1, 45}$ = 4.668, P = 0.036). High-quality males performed more courtship attempts towards females than low-quality males did (see Figure 3). This suggests that the quality and attractiveness of both the females and males influences the number of courtship attempts made by males towards females.
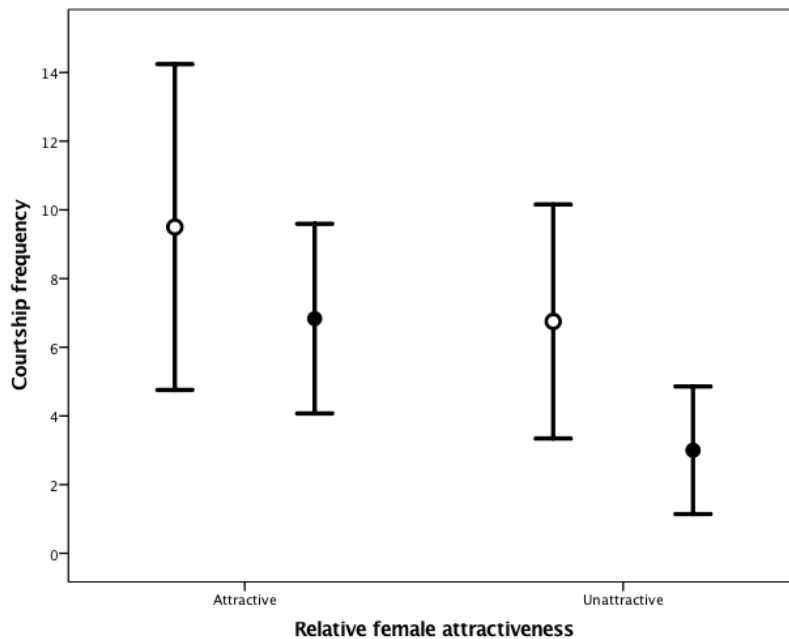
Figure 3: Courtship frequency (y-axis) in relation to sex and quality and attractiveness showing that both relative female attractiveness (x-axis) and male quality (high-quality: open circles, low-quality: closed circles) had a significant effect on courtship frequency. Attractive females received significantly more courtship attempts than unattractive females, and high-quality males performed significantly more courtship attempts than low-quality males. The error bars show the 95% confidence interval of the mean. Raw data was used in this figure, rather than the transformed data used in the statistical tests.

Discussion

Regardless of an individual's own quality and its mate's attractiveness, this study found no evidence that courtship latency and courtship frequency were dependent upon one another. The time in which a male takes to begin courting a female, does not influence the number of courtship attempts that he the proceeds to make. This is not surprising when the results from tests on courtship latency and courtship frequency are taken into account. Courtship latency was unaffected by quality and attractiveness, meaning males must base their decision of when to court on something other than quality and attractiveness. On the other hand, the decision of how much to court a female and the amount of energy males invest in courtship was affected by quality and attractiveness. This means that the males were able to assess the benefits of courting the female on her attractiveness, relative to their own quality.

Courtship latency

This study found that an individual's own quality and the attractiveness of their mate does not affect the time in which it takes a male to begin courting a female. Males of both quality levels were equal in the time which it took them to initiate courtship towards a female, with relatively attractive and unattractive females receiving the same latency of courtship advances from males. This would suggest that the decisions faced by a male when deciding if and when to initiate courtship are independent of his own quality and the attractiveness of the female.

This result can be understood with findings from Eastwood & Burnet (1977), who

conclude that courtship latency is related to age and the physiological state of the female. They found that younger males initiate courtship slower than older males. This can explain why there was no change in the courtship latency shown between the different quality levels of male, as males in this study were all of relatively the same age. Shorey & Bartell (1970) proposed, with further support later from Averhoff & Richardson (1974), that a volatile female pheromone exists with capabilities to stimulate males to initiate courtship. Eastwood & Burnet (1977) suggests that mature virgin females produce this pheromone at a sufficient level to stimulate males to begin courtship, while immature females do not yet produce it and fertilized females do not produce enough of it. This again explains why there was no change in courtship latency, as females in this study were all of the same physiological state, as they were all virgins.

This can be further supported by results of Schultzhaus et al. (2017), who measured courtship latency in competitive mating assays between males and females of different quality levels, manipulated by varying levels of nutrients. They found no difference in courtship latency in both female and male choice assays when presented with two individuals of varying attractiveness. It would seem that quality and attractiveness does not influence courtship latency, with male age and female physiology being the drivers of courtship initiation.

In field populations, females are often approached by multiple courting males simultaneously. Females will often accept the male who courts first (Pekkala et al., 2009), with males who are slower to court being out competed by their rivals (Eastwood & Burnet, 1977). As older males have been found to court faster and therefore be accepted more, it seems reasonable to assume that the age of the males is what determines their attractiveness to females. Results from Long et al. (1980) support this idea, as females were found to accept mating from older males significantly more than younger males. This preference by females for older males can be justified by findings from Kvelland (1965) which show an increase in fertility of males as they age, and therefore increasing the mating benefits to the female.

Although it seems that courtship latency is unaffected by quality and attractiveness in this study, individuals can use other information about their potential mate in order to determine the benefit they will receive in courting them, therefore assessing their attractiveness. For example, the way that quality and attractiveness were manipulated through altering of diet in this study does not impact upon a males age, nor does it impact upon the female's physiological state. With that being said, due to the increase of fertility that males experience with age, their attractiveness to females also increases as they age (Long et al., 1980; Kvelland, 1965). Similarly, the females physiological state can influence her attractiveness, with mature virgin females being courted faster and are more preferable to males than immature virgins and fertilized females (Eastwood & Burnet, 1977). This suggests that while results of this study suggest that quality and attractiveness does not influence courtship latency, individuals can determine the quality and attractiveness of their potential mate by assessing other traits, including their age and physiology, to influence their decisions on whether to invest in courtship. So while it may be contradictory to the results of this study, it is likely that quality and attractiveness do have an impact on courtship latency, but not through the route that was manipulated in this study.

Courtship frequency
This study found that an individual's own quality and the attractiveness of their potential mate both play a role in determining the number of courtship attempts a male makes towards a female. High-quality males performed more courtship advances than low-quality males, and high-quality females received more courtship advances than low-quality females. This shows that the amount of energy invested in courtship from the male is affected by his own quality and the attractiveness of the female.

High-quality males invested more energy in courtship activity than low-quality males did. This has been found also in other studies of similar species. Blay & Yuval (1997) found in their study of the Mediterranean fruit fly, *Ceratitis captitata*, that high-quality males had increased courtship activity, but interestingly found that low-quality males transferred a greater number of sperm than high-quality males. Droney (1998) found similar results when measuring the weight of testes in *Drosophila grimshawi*, finding that low-quality males invested relatively more of their energy into testes mass than high-quality males, who invested more in courtship activity. The study suggests that high-quality males had enough energy to invest in both of these expensive fitness-related traits, while low-quality males invested more in teste mass as a result of a trade-off decision between the two. This may have been the case in this study, but neither testes mass or sperm production/transfer were measured so it cannot be confirmed.

Females use a series of rejection movements in order to keep courting males at bay while she assesses him and his courtship advances (Eastwood & Burnet, 1977). This may explain why attractive females received more courtship advances than unattractive females as they would require higher levels of courtship to be impressed and accept the males. Both quality levels of males invested more energy into courting attractive females in order to receive the greater benefits of mating with an attractive female, compared to the energy they invested in courting an unattractive female. This shows that males alter their energy investment in courtship activity when the benefits of courting a female are altered. In some cases, they deceitfully try to seem more attractive to the female, in the case of low-quality males. The study also shows that a female's attractiveness directly relates to the number of courtship advances she receives.

The way quality and attractiveness were manipulated in this study revealed that the number of courtship attempts made by a male may have been the outcome of differing trade-off decisions towards courtship activity or testes mass/sperm production from high and low-quality males. While it cannot be confirmed from this study, results of similar studies would suggest this to be a strong possibility. Investing more energy into courtship activity is a more energetically expensive strategy, but it is the more advantageous decision as it is correlated with mating success (Droney, 1996). High-quality males may be able to afford the high energetic cost of courtship, and cope with the costs of reduced longevity from increased sexual activity by benefitting from an increased reproductive fitness. This decision to cope with the costs of an increase of courtship activity can also been seen in low-quality males when they are presented with an attractive female. This suggests that as the attractiveness of a potential mate increases, so do the benefits of courting them, therefore outweighing the higher costs incurred to an individual in order to pursue them.

Mating behaviour
This study fell short in providing an understanding of how quality and attractiveness affected mating behaviours. Information on these behaviours would have provided more detailed information on how females were affected by the attractiveness of males. However, with only three of the pairs in the study performing any mating, it was not possible to use this data in any statistical tests or carry out any useful analysis on this aspect of the study. Why there were so few incidents of mating is unknown as there seems to be no obvious explanation for this. Other studies where quality and attractiveness were altered through the manipulation of diet found mating to occur, with Schultzhaus et al. (2017) finding mating to occur across all of their treatments of diet manipulation, with the lowest percentage of successful mating being 69%. This suggests that the manipulation of quality and attractiveness, through differing diets in this study, was not the reason for this low number of mating occurrences. The flies were filmed for one hour in the mating chamber in this study, this hour would seem to be sufficient enough time for the flies to show mating behaviour. Other studies have found mating to occur within this time, with Markow et al. (1978) finding 97% of males, that were either virgin or experienced, mated within their one-hour cut-off period. Not only does this allow for the length time in which the flies were in the mating chamber to be ruled out as the reason for this result, but also that the virginity of the flies used did not affect this. It seems reasonable to assume that there must have been an error in the conditions which the flies were kept under, or in the husbandry techniques carried out in the study which led to this absence of mating, but specifics of these errors are unknown.

Conclusions
While the use of manipulating an individual's quality and attractiveness through the altering of their diet has been shown to be effective in other studies (Shelly et al., 2002; Landolt & Sivinski, 1992; Droney, 1996), there are still some limitations with it. The way in which the nutritional levels of the two diets were altered required the low- quality diet to be made with agar of higher dilution than the agar provided in the high- quality diet. This dilution poses two problems which may have had implications upon the results of this study. Firstly, it could not be known if the flies that were reared on the low-quality diet actually consumed fewer nutrients than the flies on the high-quality diet. Flies reared on the low-quality diet may have compensated for the low levels of nutrients by consuming more of the agar, with studies having found this to occur in some cases (Tatar, 2011). Secondly, the distribution of nutrients within the agar provided to the flies may not have been equally available, which may have resulted in an unequal consumption of nutrients within flies of the same diet. As well as these limitations which may have affected the results of this study, the use of diet as a mechanism of quality and attractiveness can limit the use of results when extrapolating them from a laboratory environment to a field environment, as studies have found marked differences in the phenotypes of flies reared on standard laboratory diets and field diets (Kristensen et al., 2015). While this may not have had an impact upon the results of this study, it may mean that the results may only be appropriate for comparisons to other laboratory populations rather than field populations. While this may be the case, the use of diet in this study helped reveal which aspects of courtship can be influenced by environmental factors and energy availability, and that individuals can determine the attractiveness of a potential mate based on traits which are unaffected by diet.

This study sought to understand how differing levels of quality and attractiveness affected the decisions and effort invested into courtship and mating. This was achieved in some aspects of the study, but unachieved in others. While results on mating behaviours revealed little around this area, courtship behaviours were able to be looked at in detail, revealing how various decisions that individuals face differ depending on the attractiveness of their potential mate. The benefits that an individual can receive from courting, and therefore mating with another individual, are not fixed for all. Individuals of low-quality will receive relatively greater rewards from mating with a highly attractive individual, compared to the benefits received by a high-quality individual if they were to mate with an unattractive individual. These benefits are what allow individuals to weigh up the costs of pursuing or accepting a potential mate, which influence the effort which they invest into courting and mating. Individuals use information about their potential mate to determine their attractiveness. This study showed that this information can come from a variety of traits, including some which are directly related to environmental factors and others being related to an individual's development and life history traits. An individual's overall attractiveness is the combination of multiple factors, some which are unable to be altered by the individual, and some which are able to be altered, even if deceitful to the assessor, in order to increase their fitness.

Overall, the results of this study contributes to the discussion around how organisms decide when and who to court by revealing that different aspects of courtship behaviour are influenced by different aspects of an individual's quality and attractiveness. The study shows that quality and attractiveness is not simply just related to the quality of an individual's diet, although it does play a role. Level of attractiveness is determined by multiple aspects of an individual, which can all have influences on the trade-off decisions which animals face. Therefore, this study can be beneficial to future studies aiming to manipulate quality and attractiveness. It highlights that while their results may or may not suggest that quality and attractiveness have an effect on courtship behaviours, it is important to consider that other factors, aside from ones that were manipulated, may also contribute to quality and attractiveness.

Results of this study could be extended by investigating if an individual's unattractive traits which they cannot influence, for example their age or physiological state, can be outweighed by being highly attractive in traits which they are able to influence, like courtship performance. This would reveal which kind of traits have the most influence on attractiveness, and therefore reveal what may be most important to an individual when assessing a potential mate. Understanding which traits are most important to an assessor would allow for it to be understood what they take into consideration most when making their trade-off and energy investment decisions.

Acknowledgements

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