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Variability in polymorphism and sex ratio of the conehead *Ruspolia differens* Serville (Orthoptera: Conocephalidae) in north-west Tanzania

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Abstract
We scrutinized variability in colour polymorphism and sex ratio of the cone-headed tettigoniid *Ruspolia differens* (Orthoptra: Conocephalidae) in north-west Tanzania during periods of swarms and of non-swarming phase. Six key colour morphs were characterized together with their continuous and discontinuous sub-morphs. Seasonal weather alterations evidenced to alter the relative frequency and sex-ratio biasness of these morphs. During dry season the equilibrium of colour polymorphism favours the frequency of the browns and the population is chiefly male-biased, but during wet season the greens are favoured and the population is female-biased. This implies that these traits are directly governed by weather conditions and are subjects to vary with geographical regions. Tegmina coloration showed to be a sex-linked trait as brownness was associated with maleness whereas greenness was associated with femaleness. Further, tegmen speckles are a useful biomarker of inheritance of the browns and the greens are dominant over the browns. It was apparent that the distinct variability in colour polymorphism and sex ratio of this insect is its interactive strategy for inhabiting different weather and environments.

Keywords: Polymorphism; conehead; Conocephalidae; *Ruspolia differens*.

INTRODUCTION

The African conehead *Ruspolia differens* (*senene* by its Kiswahili name) is a widely edible conehead (i.e. subfamily Copiphorinae) occurring in swarms and non-swarming phase in eastern Africa and other Afro-tropical regions (Olembo, 1970; McCrae, 1982; Dunkel, 1995; Mbata, 1995; Agea *et al.,* 2008). This insect has colour polymorphism and sex-ratio biasness (Owen, 1965; Bailey, 1979; Bailey and Rentz, 1990), but the variations in these traits are not very clearly understood although they might be significantly differing from those of the other coneheads. A number of tettigoniid species are distinguished based on their coloration profiles. *Orchelimum concinnum* is identified by its vertical facial stripe, *O. fidicinium* may be green or brown, *Conocephalus fasciatus* is largely green with a characteristic black stripe running from fastigium to tip of abdomen, and *C. spartinae* is green with an orange abdomen (Wason and Pennings, 2008). The multitude of colour morphs of *R. differens* is not specially used for diagnostic identification of this species. This is notably because the coloration status of these morphs is only partially known although it might afford useful taxonomic information.

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The available reports on sex ratio of *R. differens* are somewhat conflicting. Owen (1965), who took hand-caught samples from a white sheet beneath a mercury-vapour bulb in the Kampala swarms in 1962, reported a sex ratio of 1:1.24 in favour of the females. This ratio was relatively comparable with only one year of the five years of such sampling by Bailey and McCrae (1978) who used Robinson m-v light trap some 0.6 km from the site of Owen (1965) where sex ratio was found to be 1:1. In the rest of the other years (four) the overall count of the males was significantly higher than those of the females. The remarkable variation in sex ratio of *R. differens* has led to some authors speculating that this species comprises sexually dimorphic flight (Bailey and McCrae, 1978) and that the females are more attracted to light than the males (McCrae, 1982). However, there has been no thorough scrutiny of these assertions despite the fact that this might contribute some reliable taxonomic data. Poor knowledge of diagnostic characters of *R. differens* hampers its value as a human food item. The present study elucidates the

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variability in colour polymorphism and sex ratio of *R. differens* in north-west Tanzania. It facilitates a useful guide to identification and categorization of this insect and contributes new knowledge for promoting its consumption and conservation.

MATERIALS AND METHODS

Study site

This study was conducted in Bukoba and Muleba districts located in Kagera region between 1.00° and 2.45° S, and 30.25° and 32.40° E in the north-western corner of Tanzania (United Republic of Tanzania, 2008). These districts were among the major East African zones with immense abundance and consumers of *R. differens* (Bailey, 1979; McCrae, 1982) hence the choice as study site. Preliminary information showed that swarms usually occur in two periods: November-December and April-May, while the non-swarming phase occurs in the rest of the other months. This insect is associated with areas of bushes and is largely nocturnal and readily attracted to light. Records indicated normal weather conditions in the study site (TMA, 2009).

R. differens sampling

Composition analysis was based on *R. differens* adults. These were collected with hands mainly after turning bush grasses or shaking trees and shrubs in three villages namely Nkindo (Bukoba Urban), Makonge (Bukoba Rural) and Rwagati (Muleba). Non-swarming insects were collected almost after every two weeks in October 2008 and January, March and June 2009 (i.e. four months all close to the period of swarms). Searching was conducted in the early morning (from 07.00 to 09.00 a.m.) when these insects were largely inactive and the first 100 individuals to be encountered were collected. A total of 800 non-swarming specimens were collected from 8 different bush plots in this sampling. In addition, a total of 6,000 swarming specimens were collected from the local people in their usual routine of collecting the insect for food. These were obtained in 10 bunches (each with 100 insects) from each of the six swarming clusters which hatched (i.e. one cluster in November and in May, and two clusters in December and in April). Immediately upon collection all *R. differens* samples were categorized and recorded in order of sex and color for composition analyses. They were photographed with a JVC camera (Model GR-D770, manufactured by Victor Company of Japan Limited). A number of 100 adult specimens were killed by freezing, then were preserved dry for future reference. The rest of the samples were immediately preserved in 70% ethanol. All samples were transferred to University of Dar es Salaam (Department of Zoology and Wildlife Conservation) for further analyses.

Colour morphs

Colour morphs of *R. differens* were visually distinguished in the field following the descriptions of Owen (1965) and McCrae (1982). The coloration profiles of these morphs were distinguished in photographs based on the predominant colour, suffusions, stripes and speckles in tegmina. Their hues were rated in terms of RGB (red, green and blue) status using an RGB Colour Selector (custom bar) of Windows Vista (Home Premium) installed in a Toshiba 2009 Satellite M505-S4940 computer. Sex ratio (defined as number of males per number of females) was analyzed across the period of swarms and of non-swarming phase.

Data analysis

Consistence in colour polymorphism and sex ratio was analyzed using Chi-square (χ^2) Test of Contingency Table. Sex ratio biasness among samples was analyzed graphically upon dividing number of males by number of females (Gäde, 2002). Significance of relationship between sex ratio and colour polymorphism was tested using Pearson correlation coefficients. Similarity in sex ratio between non-swarming phase and the swarming phase was tested with Student's *t*-test. All statistics were analyzed using XLS spreadsheets of Microsoft Office Excel 2007.

RESULTS

Coloration and Colour morphs

The coloration plan of *R. differens* evidenced to comprise six key colours namely green, brown, purple, yellow, black and grey. Patterns of RGB (Table 1) indicated that: (i) the species occurs in at least six key colour morphs (forms) with a number of continuous and discontinuous sub-morphs. (ii) Tegmina are either green or brown and may comprise purple stripes or suffusion. (iii) All brown tegmina are also densely spread with dark speckles. (iv) All individuals have yellow jaws and purple clypeus (iv) Eyes are black or grey.

The study encountered five key colour morphs of *R. differens,* namely green, brown, purple striped green, purple suffused green, and purple suffused brown (Table 2 and Fig. 1) and also recognized the sixth rare form (purple striped brown) described by Owen (1969) some four years after describing the other five forms. The rareness of the sixth morph was also confirmed by local information. It was also revealed that all key colour morphs exist in various sub-morphs which notably arise from different levels of discontinuous and continuous variations indicating that the trait is controlled by intrinsic (genetic) and extrinsic

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Figure 1: *R. differens* colour morphs: (a) Key morphs (1 = Green; 2 = Brown; 3 = Purple striped green; 4 = Purple suffused green; 5 = Purple suffused brown) (b) Densely speckled tegmen of the brown form (c) Other green sub-morphs apart from a1 (c1 = purple legged green with grey eyes; c2 = green (paler) with grey eyes and scanty, faint tegmen speckles; whilst a1 = green legged green with black eyes (d) Brown sub-morphs (1 = light brown; 2 = dark brown; 3 = rufous brown).

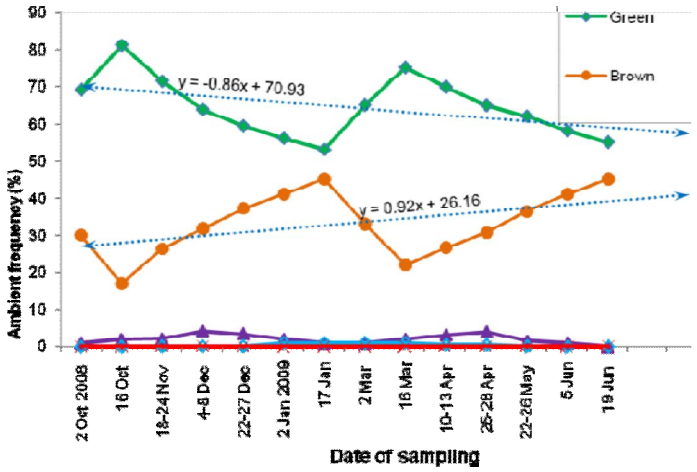


Figure 3: Temporal variation in relative frequency of key colour morphs of *R. differens* in Bukoba and Muleba, 2008/2009.

(ecological) factors. Discontinuous variation manifests in the predominant colour; presence or absence of stripes, speckles or suffusions; eye colour (black or grey); and legs colour (green, brown, or purple). Continuous polymorphism manifests in ranges of hues of body colorations as revealed by the varying SD of RGB (Table 1).

Frequency variability among colour morphs

Frequency distribution of *R. differens* morphs is summarized in Table 3. The overall relative frequency of these morphs (Fig. 2) indicated that the greens are most predominant (65%) followed by the browns (31.8%), purple striped greens (2.8%), purple suffused greens (0.2%) and purple suffused browns (0.06%). However, temporal variation in frequency of the two most predominant morphs (i.e. the greens and browns) was noted. Frequency of the greens (mimickers of fresh vegetations) was found to decrease along the swarming season with a linear average gradient of -0.86, while that of the browns (mimickers of dry vegetations) evidenced to increase along the same season with a linear average gradient of 0.92 (Fig. 3). Therefore, colour polymorphism of this insect varies with environmental changes across the swarming phase (rainy season) towards the non-swarming phase (dry season) and the greens and the browns largely define this phenology. Further, it was apparent that the frequency of the greens is largely favoured by sexual reproduction (genetic recombination) while the frequency of the browns is favoured by environmental selection. This appeared to be a strategic hallmark in *R. differens* evolution.

Inter-seasonal stability of colour polymorphism

Analysis of long-term variation in relative frequency of key colour morphs of *R. differens* was done based on a number of 6,800 specimens. Therefore, the expected frequency (comprising 6,129 insects) for this analysis was obtained by multiplying all values of the comparable frequency (Owen, 1965) by a factor 6800/6129. Based on χ^2-test it was found that p=0.23 hence >0.05. It was concluded that the overall frequency of major colour morphs of *R. differens* does not significantly differ from how it was reported some 45 years ago. The long-term stability of colour polymorphism is a justification that this trait is largely genetically determined. The results are also evidencing that the females of this species are not necessarily more readily attracted by mercury vapour light than the males. This is notably because the relative frequency of the colour morphs in bushes (as ascertained by this study) is comparable with that of the morphs collected at mercury vapour light by the previous workers.

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Intra-seasonal stability of colour polymorphism

The present study also focused on temporal variation in colour polymorphism of this insect in six consecutive swarms to understand the intra-seasonal stability of this character. The mean frequency of each colour morph was calculated for the overall swarms and the resultant distribution was taken to be the expected frequency for stability analysis. Only the second swarm ($p=0.2$) and the fifth swarm ($p=0.3$) did not deviate from the expected frequency as $p>0.05$ (Table 4). This is notably because both these swarms are the peaks of the swarming capacity of this insect hence they comprise the typical characters of the swarming phase. The rest of the other swarms deviated from the expected frequency most likely because they are intermediately positioned between non-swarming phase and the peak of the swarming phase.

Sex-ratio variability among morphs

Sex ratio is a result of selfishness of certain genetic elements and is one of the key parameters of evolution and ecology of organisms hence may provide useful taxonomic information. The present study ascertained that sex ratio of *R. differens* varies among the colour morphs of this insect (Table 5). Only the brown morph was found to be male-biased (mean sex ratio ≈ 2.3). All the green coloured morphs (i.e. the greens, purple striped greens, and purple suffused greens) are female-biased (mean sex ratio ≈ 0.5). However, the purple suffused browns show to be intermediate of all morphs as they have unity sex ratio (i.e. number of males ≈ number of females) (Fig. 4).

Temporal dynamics of sex ratio

Sex ratio of *R. differens* was found to vary across time frame of life cycle of this insect (Fig. 5). At the beginning of the swarming phase, sex ratio << 1 hence much female biased. With time, however, the number of males approaches the number of females to almost unity (i.e. 1) sex ratio by the end of this phase. During the non-swarming phase the males outnumber the females (i.e. sex ratio > 1), but as nymphs emerge into swarms sex ratio favours the females again (i.e. sex ratio << 1) and the cycle continues. The trend implies that genetic recombination (sexual reproduction) favours the females, while environmental selection favours the males. The overall sex ratio of *R. differens* was approximately unity (i.e. 1) indicating that the number of the females almost counterbalances that of the males. The vast abundance of the females at the beginning of the swarming phase when also weather conditions are largely favourable and the resources are widely abundant appears to be a strategy of this species to maximize reproduction in this period. On the other hand, sex ratio favours the males only during the season of scarcity of resources when weather is largely unfavourable. This strategy is notably intended to minimize reproduction during the periods of unfavourable conditions.

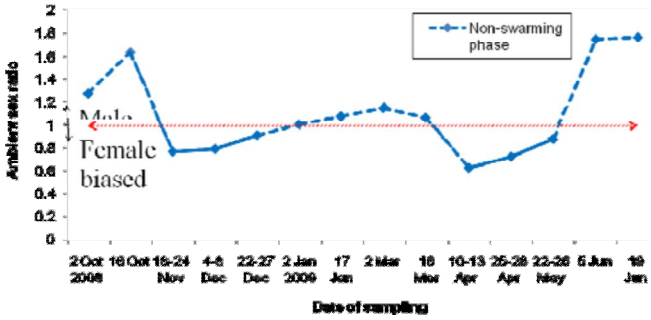


Figure 5: Temporal dynamics of sex ratio of *R. differens* in Bukoba and Muleba, 2008/2009.

Relationship between sex ratio and colour polymorphism

Analysis of this relationship (Table 6) indicates that only the frequency of the brown morph is positively correlated with sex ratio biasness (r = 0.9). The frequency of the greens (r = -0.9) and that of the purple suffused greens (-0.4) are negatively correlated with sex ratio biasness. The frequency of the purple striped greens and that of the purple suffused browns are not correlated with sex ratio (r≈0) indicating to lie between the greens and the browns. A differential selection between the greens and the browns was apparent. During the non-swarming phase the browns (the most male-biased) are more selected than the greens (the least male-biased). However, the greens (the most female biased) are returned into predominance by genetic recombination (sexual reproduction) as evidenced in the emerging adults. This clearly implies that green coloration is genetically dominant over brown coloration and that post-embryonic selection favours the browns over the greens.

Comparison between sex ratio biasness of non-swarming phase and of the swarming phase

Analysis (based on Student's t-test) of difference in sex ratio between the two phases (Table 7) ascertained that p=0.01 hence <0.05. Therefore, sex ratio biasness significantly varies between non swarming phase and swarming phase. During swarms (wet season) females outnumber males notably to maximize fecundity in this period of optimum resources. Conversely, males outnumber females during periods of non-swarming phase (dry season) implying an adaptive strategy to minimize fecundity during scarcity.

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DISCUSSION

Variability

The findings of this study concur with the observations of earlier workers (Owen, 1965; Bailey and McCrae, 1978; McCrae 1982) that *R. differens* comprises at least six key colour forms each with a number of sub-morphs which are controlled by genetic and environmental forces. The mode of polymorphism of this insect receives special attention due to its seasonal variation with the environmental conditions. The species is very well adapted to vary the frequency of its colour morphs in order to camouflage itself with the environmental conditions and utilize them efficiently, but the overall number of the greens per season almost balances with that of the browns. This pattern is rather unfamiliar because in other coneheads only one colour morph is consistently predominant. Despite all variations, the colour morphs of *R. differens* appear to be conspecific. *R. differens* is very well adapted to its indigenous environment. It has strategically learnt to cope with the environmental changes by seasonally varying the frequency of its colour morphs and the bias of its sex ratio. The environmental conditions are favourable during rainy season hence the greens and the females predominate to enable the species camouflage itself in green vegetations and reproduce effectively. However, during dry weather (unfavourable conditions) the browns and the males predominate to enable the species camouflage itself in dry vegetations and minimize reproduction. The observation was also supported by local information which clarified that the swarming season is characterized by conspicuous predominance of the browns coupled by critical shortages of the greens. This pattern had not been clearly described.

Phylogenetic implications

The findings provide useful information on the phylogenetic affiliation of *R. differens.* The key colour morphs of this species are largely genetically determined. Occurrence frequency of the greens is chiefly promoted by genetic recombination (sexual reproduction) with almost a corresponding reduction in frequency of the browns. Rather, the frequency of the browns is promoted by post-embryonic selection with almost a corresponding decrease in frequency of the greens. This implies that the greens are genetically dominant over the browns and that the browns are better adapted for the post-embryonic life in the study site than the greens. The colouration patterns of *R. differens* appears to be a sex linked character as brownness is mostly associated with maleness while greenness is associated with femaleness. Tegmen speckles are useful genetic biomarkers of the inheritance of the brown forms because all morphs with this trait (the browns and the purple suffused browns) were also densely speckled. The lightly speckled sub-morphs might be resulting from incomplete dominance. The genetic link to the purple suffused trait is unknown; nonetheless, the genetic attributes of all these traits are being constructed elsewhere in an allied study.

Biosystematics

Colour polymorphism might be a useful tool for distinguishing *R. differens* from other species, but also among individuals of different geographical affiliations hence their sub-categorization. As with other widespread species of the genus *Ruspolia,* this insect could be composed of at least three subspecies based on the main areas, West Africa, Central Africa and the Rift valley, and South-east Africa and the shore islands, but it has been impossible to conclusively subdivide this extant species without getting ample evidence from live specimens (Bailey, 1976, 1979). Because the frequency of colour morphs of *R. differens* varies with environmental conditions, any two or more different environments essentially comprise correspondingly different frequencies of the morphs. The variation can be a useful tool for species sub-categorization. The new findings immediately attracts a comparison with the known other species which have the tendency to swarm. Under low-density, locusts and the Mormon crickets occur in solitary predominantly comprising the green individuals. These are aposematic insects which characteristically avoid associating one another. An increase in physical contacts among the individuals stimulates gregarization in which the insects switch into gregarious phase. They become brightly black patterned yellow, more active and easily attracted to one another to form aggregations that can grow into swarms. The switching between swarms and the solitary phase occurs within 4 hours of crowding or isolation, but the colour changes occur gradually over a matter of several developmental stadia (Bailey *et al,* 2005). The swarming of locusts and the Mormon crickets is usually accelerated by shortage of resources. Conversely, *R. differens* is unique in that it develops swarms only during seasons of ample resources. The individuals lack aposematism as they do not change colour or avoid one other particularly during non-swarming phase. All these traits largely characterize *R. differens,* but their ancestry is not clearly understood hence requires further attention.

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