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CHROMATOSOMES IN THREE PHENOTYPES OF *NEOCARIDINA DENTICULATA* KEMP, 1918: MORPHOLOGICAL AND CHROMATIC DIFFERENCES MEASURED NON-INVASIVELY

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ABSTRACT

External colour differences in crustaceans were examined using a non-invasive study of morphology and colour in epidermal chromatosomes. Three different phenotypes of the shrimp, *Neocaridina denticulata* (< 2 cm total length), differ in the quantity and dispersal of chromatosomes in the epidermis, with resulting differences in body colour. Also, pigmentation pattern is most pronounced in the anterior body parts. The exopod of uropod was the most suitable body area to monitor colour changes in this shrimp. This non-invasive method allows for the study of external pigmentation in crustaceans at different stages and may help to understand how these animals manipulate their chromatosomes under physiological and environmental variability in order to acquire the final external colour.

KEY WORDS: chromatosome, colour, digital imaging, morphology, *Neocaridina denticulata*

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INTRODUCTION

The freshwater red cherry shrimp, *Neocaridina denticulata* (Kemp, 1918), in Atyidae, is widely distributed in Asia (Hung et al., 1993; Englund and Cai, 1999). External colour of this species is quite variable and its exoskeleton is somewhat transparent due to its weakly calcified integument (Hung et al., 1993) and low level of sclerotization as in other caridean species (Bauer, 2004; Noël and Chassard-Bouchaud, 2004).

In many crustaceans and fishes, the perceived external pigmentation is due to the physical phenomena of absorption, scattering and reflection of light over the body surface (Noël, 1983; Fujii, 1993). Regarding the absorption and reflection of light, the distribution of pigmented cells named chromatophores contributes in great extent to the final body colour and patterns perceived (Miner et al., 2000; Oshima, 2001). These cells are mononucleate and have a ramified and flattened shape with long extensions, contending pigmented granules (Noël et al., 1983; Fingerman, 1985). In many decapod crustaceans, these chromatophores are clusters of up to 20 individual cells and organized in colour units called chromatosomes (Elofsson and Kauri, 1971) and when located at the epidermis these chromatosomes form the basis of any chromatic arrangement (Noël and Chassard-Bouchaud, 2004; Boyle and McNamara, 2005).

External colour change in crustaceans is mainly due to dispersal and aggregation of pigments in the ramifications or a change in chromatophore density (Tume et al., 2009); nevertheless, the speed at which these changes take place is the result of a morphological or a physiological adaptation (Noël and Chassard-Bouchaud, 2004). Morphological adaptation relies on change on the net amount of pigment and quantity of cells on the skin and operates in the long term. Meanwhile, physiological adaptation is based on motile activities of pigments inside the chromatophore extensions and operates in the short term (Fujii, 1993; Noël and Chassard-Bouchaud, 2004). While control mechanisms and kinetics of the dispersal and aggregation have been studied (Nagabhushanam, 1969; McNamara and Taylor, 1987; McNamara and Ribeiro, 1999), quantification of both morphological and chromatic characteristics of chromatophores (or chromatosomes) is understudied despite the potential contributions of both in the final colour expression.

Although in general most atyid shrimp are model species for scientific research due to their ease of handling and maintenance under laboratory conditions (Jalihal et al., 1994), some species are so small that impedes the application of standard physical or chemical methods for pigmentation or skin colour assessment (Flores and Chien, 2008). Digital Image Acquisition and Processing (DIAP), is a viable method for use on small species like *N. denticulata*. In this method, the desired area of the sample is photographed and processed using computer software (Luzuriaga et al., 1997), such as in the analysis of colour change and pigmentation in aquatic species, including fish and crustacean (Hatano et al., 1989; Luzuriaga et al., 1997; Wallat et al., 2002; Wallat et al., 2005), thus it is also suitable for analysis of colours in the visible spectrum (Gerald et al., 2001). This non-invasive approach may also be useful for identification of species during larval stages (Kikkawa et al., 1995) and the assessment of pigmentation changes, related to life histories.

Fig. 1. Three different phenotypes of *Neocaridina denticulata* used in the study. From left to right: red, reddish and translucent.

Herein, we non-invasively measure some morphological and chromatic characteristics of chromatosomes as a colour unit, to analyse their contribution to the external pigmentation in the epidermis in three colour phenotypes of a freshwater shrimp.

Materials and Methods

Experimental Design

A single batch of red cherry shrimp from the Kuo-Min Liu's ornamental fish farm in Pingtung county, southern Taiwan ROC, was used in this study. In the farm, the shrimp were kept in a cement pond where they fed ad libitum on algae of the family Oscillatoriaceae. Only adult females were used to avoid variation due to sexual dimorphism or age because colour may be affected by developmental stages and sexes in crustaceans (Meyers and Latscha, 1997; Noël and Chassard-Bouchaud, 2004). The experimental animals (mean total length = 15.92 ± 1.08 mm, mean carapace length = 4.33 ± 0.37 mm, $n = 43$) were separated into three colour groups: red (D, $n = 13$), translucent (T, $n = 15$) and reddish (R, $n = 15$), following colour classification in Hung et al. (1993) (Fig. 1). In each shrimp, chromatophores of three specific body parts on the right side were analysed: over the branchial region of the carapace (C), the pleuron of the second pleomere (SM), and the exopod of the uropod (E). To avoid pseudoreplication, a single shrimp was sampled only in one body part at a time, allowing five different shrimp to be sampled per each body part except in the group D, in which for carapace area only 3 shrimps were sampled.

Measurements

Before observing chromatosomes, each shrimp was acclimatized during 10 minutes in a 500 ml plastic beaker filled with freshwater upon a black background and under a white light at 1852-1857 lux from a fluorescent bulb (Model PL-S 13 W/865 5000°K, Phillips Electronics, Netherlands). This procedure was considered necessary to provide similar illumination and background conditions for pigment dispersion over the shrimp's body surface (Ribeiro et al., 2004). The light intensity for acclimatization was checked by a digital light meter (Model LM-81LX, Digital Instruments, Taiwan, ROC). Each live shrimp was then carefully placed upon a 76 × 26 mm glass micro slide (Kimble Micro slides, Owens-Illinois Co., U.S.A.) and covered with a translucent plastic film to immobilize it and then transferred to an optic microscope (ECLIPSE E400 bright field, Nikon Corporation, Japan). A 4× amplitude objective (Series CFI_{60} Plan Achromat, Nikon Corporation, Japan) was used.

The microscope illumination was provided by a 6 V 30 W halogen lamp (Model 5761, Phillips, Netherlands), covered by a colour balancing filter (NCB11, Nikon Corporation, Japan). Standard conditions for observation of each shrimp were secured by means of adjusting the illumination control of the microscope to the natural daylight colour tone option, and using an observation-photographic time period between 35-40 seconds. This time was considered appropriate after several previous trials, therefore, if more than this time was necessary due to difficulties in focusing the desired area during the sampling period, then the data collected was considered biased and was not included in the analysis.

Image Acquisition and Processing

A digital camera (MOTICAM 2000 USB 2.0, Motic China Group Co. Ltd, China PR) was attached to the vertical tube adapter of the microscope and connected to a desktop computer. The view field of the microscope was previously scaled using the calibration tool of Motic Image Plus V 2.0 and a micro slide. A set of multi-focus pictures of each body part was captured and stored with a resolution of 1600 × 1200 pixels in TIFF (Tagged Image File Format). This format defined the necessary tags so further colour and image processing would be possible (Süsstrunk et al., 1999).

Helicon Focus V 3.2 Pro software (Helicon Soft Ltd., Ukraine) combined each set of multi-focus pictures and constructed a final image that was processed using Video TesT Morphology V 4.0 (Video Test Ltd., St. Petersburg, Russia). A threshold option of the software characterized the chromatosomes in the image and separated them from the background. No filters and edition tools were used to keep the original image colour intact. After the chromatosome boundary was manually delineated and corrected, a data table for each chromatosome was generated with the desired parameters.

Parameters

Standard image domain was set at 2.5 mm^2 circular area in each body part. Minimal size for each identifiable chromatosome was set at 25 µm to avoid incorrect characterization.

Four morphometric parameters within the standard image domain were calculated:

CA – Cell area, the average area of each chromatosome in μm^2.
CDI – Cell dimension, the average dimension (length × width/2 in µm) of each chromatosome (Video Test, 2002).
CD – Cell density, the number of chromatosomes in 2.5 mm^2.
Fk – The average shape circle factor of each chromatosome, which characterizes the proximity of the object to a circle. An ideal circle has Fk = 1. The lower the Fk value, the more dispersed is the chromatosome. Fk can easily characterize nearly circular elements and make simple comparisons among elements of irregular shapes (Epstein et al., 1984; Cooper et al., 1994). Therefore, it was used as an index to signify the degree of dispersion of the chromatosomes (Fig. 2).

Three colour parameters based on CIELab colour space, previously used to assess pigmentation in aquatic animals (Buttle et al., 2001; Gouveia et al., 2003; Melville-Smith et al., 2003; Kalinowski et al., 2005), were calculated after appropriate transformations under standard theoretical conditions, based on the RGB values obtained from Video Test Morphology for each chromatosome (Fig. 3):

L – Brightness, representing the attribute according to which a sample appears to reflect a greater or smaller fraction of the incident light, ranging from 0 for black and 100 for white,
CH – Chroma, corresponding to the strength of colour near the dominant wavelength at any illuminated level. Using a and b chromaticity

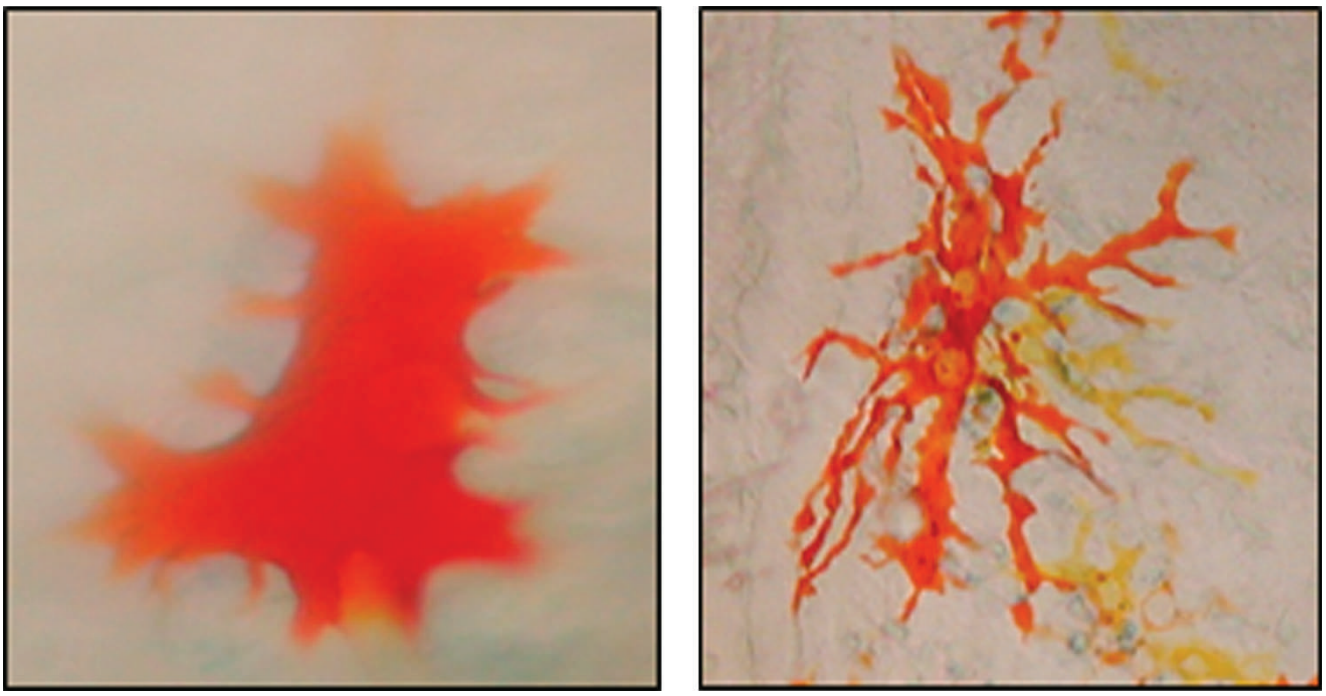

Fig. 2. Example of dispersion of two chromatosomes on the epidermis of the analysed shrimps. The chromatosome on the left (pigment concentrated) has an Fk ~ 1, while the chromatosome on the right (pigment dispersed) has Fk ~ 0. Pictures are not at scale.

coordinates (+a is defined as red, −a as green, +b as yellow and –b as blue), the Chroma was calculated using the following equation:

$$CH = \sqrt{\left((a)^2 + (b)^2\right)}$$

H – Hue (H = $\tan^{-1}$ a/b), represents the attribute according to which a sample appears to be similar to one or a mixture of two of the perceived colours red, yellow, green and blue (Choudhury, 2000). H values increase when the hue of the colours change, for example from red (H = 0°) towards yellow (H = 90°).

The values of these three parameters are easier to correlate with the visual appearance of a colour to a human eye, which possesses three types of colour sensors. Other systems using only two-dimensional chromaticity coordinates, e.g., CIE 1931 or CIE RGB, are less suitable for colour perception (Choubert et al., 1997; Ohta and Robertson, 2005).

Greater variation among individuals in the body parts analysed was considered more sensitive to change in chromatosome colour and morphology. Here the coefficient of variation was used as a measure of sensitiveness.

Some criteria were established to select the area of the body most suitable for monitoring chromatic change. For example: flatness (the flatter the better), clearness (i.e., transparent with little overlaps due to underlying organs and tissues); ease of identification and photography; harmless (i.e., causing minimal stress to the animal). To qualify each of the criteria, a qualitative ranking was established as follows: good (+) the area meets the criteria; medium (+/−) the area partially meets the criteria; and poor (−) the area does not meet the criteria established.

Statistical Analysis

A factorial design was used. Three shrimp colour groups and three body parts of theirs were assigned as the factors. Mean values of the parameters measured in each shrimp were used for the analysis. To meet parametric assumptions, CD, CDI and CA were transformed by obtaining the $\log_{10}$ of each variable. An arcsine square root transformation was used before processing Fk values, which had ratio values between 0 and 1.

Differences among treatments were determined by two-way ANOVA, with and without interaction and the significant effects compared by Tukey's studentized range test. The coefficient of variation of each parameter was used as an indicator of sensitiveness in the selection of the monitoring area. The level of significance was set at $p \leq 0.05$. Analyses were conducted using the SAS V9.0 (SAS Institute Inc., U.S.A.).

Results

Colour Groups and Body Parts

By looking at the chromatic and morphological characteristics of chromatosomes we tested the hypothesis that

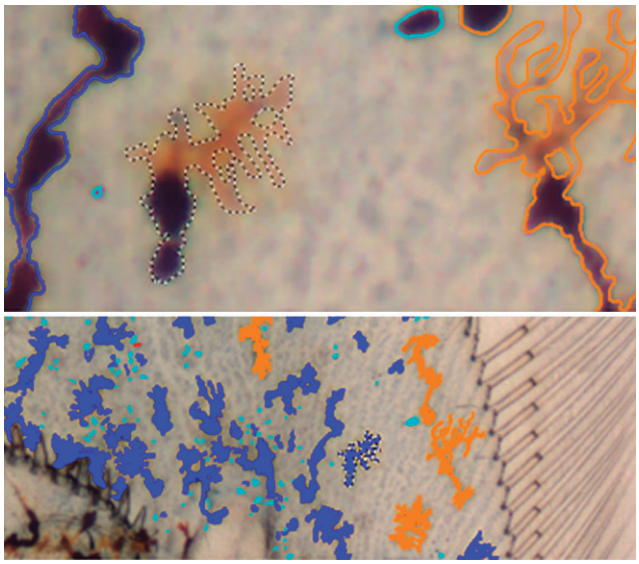

Fig. 3. Example of the use of Video Test Morphology V 4.0, to obtain morphological and chromatic parameters for each chromatosome. Highlighted a chromatosome of the exopod of a reddish shrimp with Fk = 0.10.

external colour can be effectively described by the morphology and colour of chromatosomes. The red shrimp had the largest chromatosome area (CA) and chromatosome dimension (CDI), followed by the reddish and translucent shrimp, although CA was similar in reddish and translucent shrimp (Fig. 4). Chromatosome density (CD) in the red shrimp was similar to that in the translucent shrimp but less than that in the reddish shrimp. The average shape circle factor of each chromatosome (Fk) was greatest in the translucent shrimp, followed by the reddish shrimp and then the red shrimp. Lightness (L) was lower in the red shrimp than in the reddish shrimp but similar to that in the translucent shrimp. Hue (H) was greatest in the translucent shrimp and was similar in the red and the reddish shrimp. The red shrimp had the greatest Chroma (CH), followed by the reddish shrimp and then the translucent shrimp (Fig. 5).

The carapace showed the greatest CA and CDI, followed by the pleomere and the exopod. On the contrary the CD in the exopod was the greatest followed by the pleomere and the carapace (Fig. 4). The Fk factor was greater in the exopod than in the carapace but equal to the pleomere. L was not different among the three body parts. The H values in the carapace were greater than the pleomere and the exopod. The pleomere had the greatest CH, followed by the carapace and then exopod.

CH and H were affected not only by the main effects of colour group and body part but also by their interaction (Fig. 5). In this case these two variables appear to be a function of the phenotype, as should be expected but also of the region of the body which is being considered.

Selection of Area for Monitoring

Uniformity of external colour changes may differ along the entire body of the shrimp because the chromatosomes in different areas are more sensitive in terms of chromatic and morphological changes than in others. The calculations of the coefficient of variation (CV) showed that exopod was the most variable body area with the greatest CV for the seven parameters, while the pleomere area was less variable (lower CV) for most of the parameters (Table 1). Exopod was very sensitive in CDI, CA, CH, and H and fitted all the qualitative criteria established for the selection (Table 2).

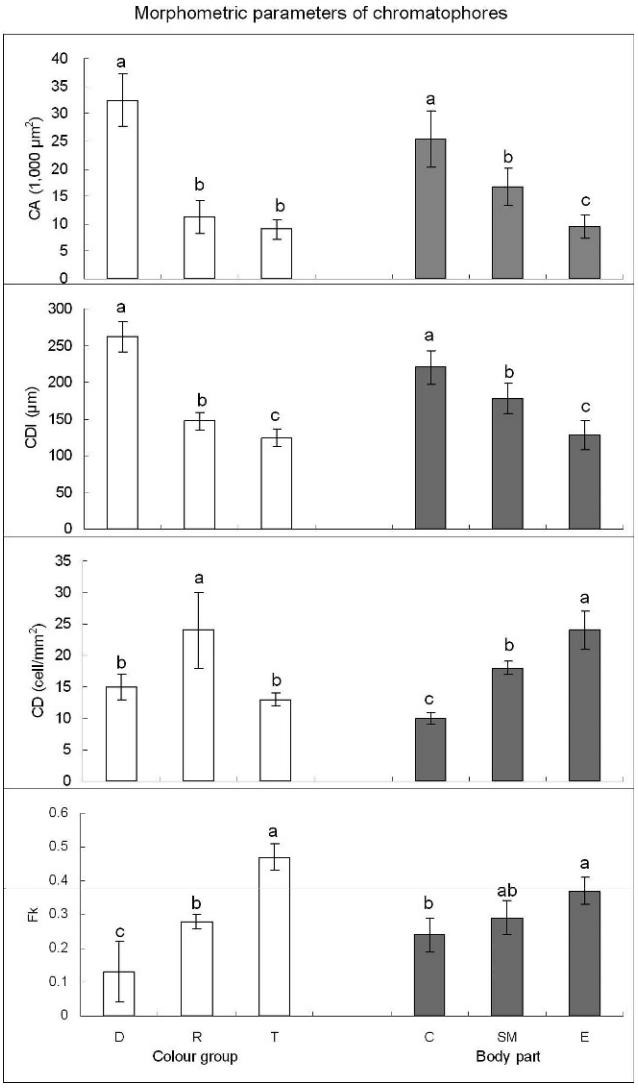

Fig. 4. The effects of colour group: red (D), reddish (R) and translucent (T) and body part: carapace (C), pleuron of the second abdominal pleomere (SM) and exopod of uropod (E) on morphological parameters: cell area (CA), cell dimension (CDI), cell density (CD) and shape circle factor (Fk) of chromatosomes of *Neocaridina denticulata*. Values are means ± S.E.M. $n = 43$. Means without a common letter are significantly different ($p \leq 0.05$).

Discussion

We found that chromatosomes can readily be examined non-invasively in small shrimp, and that differences in external colour of phenotypes can be reliably described quantitatively based on precise morphological measurements and quantitative colour values of chromatosomes. Several studies have assessed the morphology and colour of chromatophores and chromatosomes in many fishes and crustaceans (Table 3). However, early studies of those provide qualitative description rather than quantitative data, perhaps due to the lack of more precise and sophisticate tools (but see Bauer, 1981; Brown, 1935). When using dispersion-aggregation index, Hogben and Slome (1931) assigned scores for the pigments completely dispersed or completely aggregated but the definition of precise reproducible scores for the levels in between were some way subjective. In early studies on the factors associated with the expression of colour morphotypes and control of colour change of crustaceans and caridean shrimp in particular, the perceived change in wavelength of reflected light was used as the corresponding qualitative change in colour and the dispersion or aggregation of chromatosome (or chromatophores) (Gamble and Keeble, 1900; Perkins, 1928; Brown, 1935; Chassard-Bouchaud, 1965; Bauer, 1981, 1982). More recent studies of colour in aquatic animals used quantitative colour definition values to evaluate the skin colour of a whole animal, such as two-spotted gobies (Wallat et al., 2002) and gold fish (Svensson et al., 2005). Meanwhile Tlusty (2005) applied digital

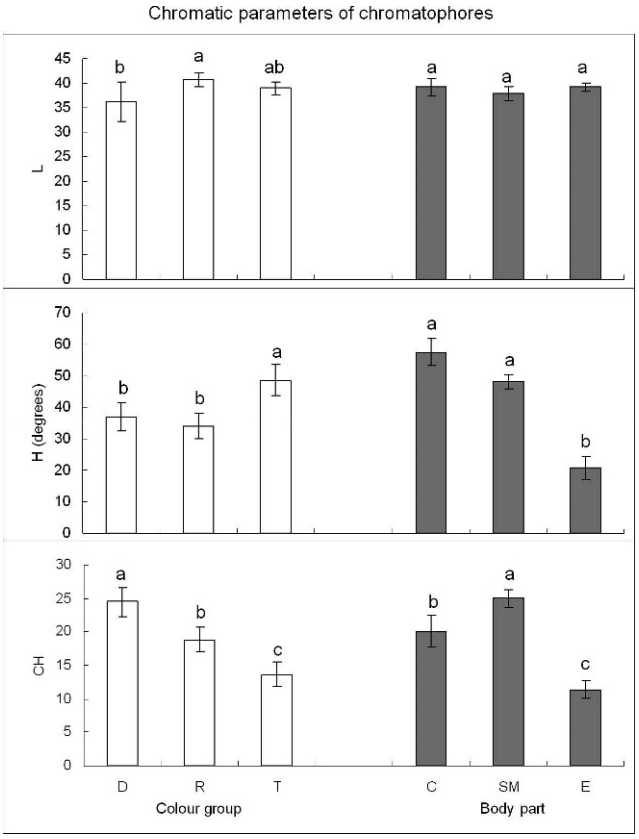

Fig. 5. The effects of colour group: red (D), reddish (R) and translucent (T) and body part: carapace (C), pleuron of the second abdominal pleomere (SM) and exopod of uropod (E) on chromatic parameters: Lightness (L), Chroma (CH) and Hue (H) of chromatosomes of *Neocaridina denticulata*. Values are means ± S.E.M. $n = 43$. Means without a common letter are significantly different ($p \leq 0.05$).

macro-photography to assess the body colouration of adult American lobster (*Homarus americanus* H. Milne Edwards, 1837).

Although with naked eyes, the external colour appears to have little change; patterns of chromatosome distribution and pigmentation vary greatly all over the body in this species. In crustaceans, chromatophore distribution may vary with the body parts and produce differences in colour patterns (Bauer, 1981; Kalinowski et al., 2005; Svensoon et al., 2005; Geiger and Bert, 2006). The occurrence of a given pattern in a population may be related to the diversity of habitats they have encountered, being some polymorphic species able to successfully exploit different substrates

Table 1. Coefficients of variation of the parameters measured in three body parts of *N. denticulata* ($n = 43$). CA = cell area, CDI = cell dimension, CD = cell density, Fk = shape circle factor, L = Lightness, CH = Chroma, H = Hue, C = carapace, SM = pleuron of the second abdominal pleomere, E = exopod of uropod.

	Parameters						
	CA	CDI	CD	Fk	L	CH	H
Body part							
C	75.21	38.98	39.01	77.70	17.42	44.10	31.91
SM	75.27	43.64	30.10	60.36	14.10	20.75	16.63
E	82.57	43.65	40.75	43.84	8.18	44.84	61.70

Table 2. Evaluation of the qualitative selection criteria established for the three body parts analysed. The symbols represent: + good ± medium − poor. C = carapace, SM = pleuron of the second abdominal pleomere, E = exopod of uropod.

Criteria	Body part		
	C	SM	E
Flatness and clearness	−	±	+
Ease of identification	−	−	+
Precision and standardization	−	±	+
Harmless	−	±	+
Sensitiveness	±	−	+

(Bauer, 1982), while others are more restricted to the ones they can resemble (Chassard, 1956). In caridean shrimp, camouflage is considered the primary function of chromatic adaptation to different habitats and background, rendering the animal cryptic to potential predators (Noël, 2000; Bauer, 2004). In its native range, *N. denticulata* inhabits different freshwater habitats, including mountain streams, ponds, reservoirs and channels in agricultural fields (Cai, 1996). Although our experimental animals came from a single fish farm, this population could have been originally collected from various places, including habitats along Taiwan through elapsed time. Other factors influencing chromatic change in this species may be linked to seasonality and maturity (Bauer, 1981, 1982; Knowles and Callan, 1939). Our experimental animals were all ovigerous females with almost the same size and were analysed during the summer, the same week after purchased, therefore avoiding differences related to sex or age. Chromatic changes may also occur as products of adaptation to differences in temperature (Smith, 1930; Brown and Sandeen, 1948), light conditions (Noël and Chassard-Bouchaud, 2004) and as direct response to UV radiation (Schmit, 1968; Miner et al., 2000; Gouveia et al., 2004; Ribeiro et al., 2004). We found in all shrimp that the chromatosomes in the carapace were larger and more dispersed than in the other regions. One explanation for this could be the protective role that these pigment bearing cells may have against tissue damage due to UV radiation or photo oxidation in general, in parts of the body where important internal organs are allocated, e.g., hepatopancreas and ovaries. Nevertheless, we are not unaware of the other functions to explain these differences, for example, thermoregulation and interspecific communication in this species, which demand further research.

Usually greater chromatophore density (CD) is associated with stronger net colouration (Sugimoto, 2002). However, our results showed that in areas where chromatosomes were more abundant, their Chroma (CH) attained a lower level (Figs. 4, 5). During our analysis the shrimp exhibited physiological colour change, possibly caused by pigment dispersion (Bauer, 1981), which was induced only by the light intensity during the pre-acclimation period. The shrimp were not subjected to any long-term treatment that could cause a morphological change. Nevertheless, as indicated previously, the study animals originally may come from different habitats so that they were adapted to a specific environment, inducing a pre-existing morpholog-

Table 3. Application of morphological and chromatic parameters in previous studies related to chromatosome/chromatophore (C) or other (O) tissues in fish and crustaceans. CA = cell area, CDI = cell dimension, CD = cell density, Fk = shape circle factor, L = Lightness, CH = Chroma, H = Hue. [a] Subjective measurement scale for chromatophore dispersion used.

Parameters		Fish	Crustacean	Source references
Morphological	CA	–	C	Miner et al., 2000
	CDI	–	C	Bauer, 1981; Boyle and McNamara, 2005; Brown, 1935; McNamara, 1981; McNamara and Taylor, 1987; McNamara and Ribeiro, 1999
	CD	C	C	Bauer, 1981; Sugimoto et al., 2000
	Fk	C	C	Amiri, 2009[a]; Bauer 1981; Brown, 1935; Brown and Sandeen, 1948[a]; Highman and Hill 1977[a], Hogben and Slome, 1931[a]
Chromatic	L	O	O	Bjerkeng et al., 1997; Buttle et al., 2001; Chatzifotis et al., 2005; Einen and Skrede, 1998; Einen and Thomassen, 1998; Gouveia and Rema, 2005; Gouveia et al., 2003; Kalinowski et al., 2005; Melville-Smith et al., 2003; Skrede et al., 1989; Skrede et al., 1990; Svensson et al., 2005; Tlusty, 2005; Wallat et al., 2005
	CH	O	–	Chatzifotis et al., 2005; Kalinowski et al., 2005; Tume et al., 2009
	H	O	C/O	Bauer, 1981; Bauer, 1982; Brown, 1935; Chassard-Bouchaud, 1965; Chatzifotis et al., 2005; Gamble and Keeble, 1900; Geiger and Bert, 2006; Kleinholz and Welsh, 1937; Kalinowski et al., 2005; Perkins, 1928; Skrede et al., 1989; Skrede et al., 1990

ical change, altering the density, kind, and location of chromatosomes of each phenotype (Gamble and Keeble, 1900; Chassard-Bouchaud, 1965; Bauer, 1982). Another factor to consider here is pigment deposition, since pigments have to distribute in different regions in the body of crustaceans, e.g., ovaries, digestive gland, compound eyes (Noël and Chassard-Bouchaud, 2004) and at different concentrations (Choubert, 2001; Nakkarike et al., 2005), causing a relocation of pigment driven by the adaptation to background conditions of each particular habitat.

Although the three colour groups (red, reddish, and translucent) presented differences in the strength of the colour (CH), the red and the reddish shrimp presented similar colour hues (H) (Fig. 5). In carideans, carotenoids are the main pigments associated with external colour (Noël and Chassard-Bouchaud, 2004). These molecules are responsible for the yellow, orange, and red colours in many carideans (Bauer, 2004) and when chemically bounded with proteins they may extend their reflection wavelength to blue, green, brown, black, or violet (Meyers and Latscha, 1997). Nevertheless, in the same chromatosome complex, there can be different classes of chromatophores bearing different pigments (Schmitt, 1968; Noël and Chassard-Bouchaud, 2004). This is especially true in caridean chromatosomes, where depending on the differential dispersion of the chromatophores into the complex, its pigment or a mixture of pigments will define the colour of the body region and produce variation in colour pattern (Bauer, 1981). In crustaceans, not all individuals of the same species can incorporate the same amount of pigments into the integument, mainly because they have to acquire it through their diet (Menasveta et al., 1994; Meyers and Latscha, 1997; Pan et al., 2001) and individual differences may exist at the genetic level. For example, Bauer (1982) found high polymorphism in populations of caridean shrimps of the genus *Heptacarpus* (Holmes, 1900) due to genetic variation. Thus, these changes appeared to be an adaptation against predators in the habitat. In the wild, intraspecific genetic variation in pigment contents has been reported in marine shrimps (Yanar et al., 2004) and in salmonids (Choubert, 2001). These genetic differences may preclude pigment biosynthesis and deposition, control of chromatophore dispersion, and chromatophore production, allowing distinct colour phenotypes to appear in the population. The environment conditions (background habitat) are known to induce morphological change that can lead to change in the intensity (chroma) of colour (Bauer, 2004). A classic example is the flexibility of morphological colour change in the shrimp *Hippolyte varians* Leach, 1814 (Gamble and Keeble, 1900; Chassard, 1956). The three colour phenotypes *N. denticulata* in our study show differences in colour hues that may be the direct consequence of differences in colour gene expression in wild animals, but can also be the consequence of morphological colour change due to environmental conditions.

Our study demonstrates that differences in colour phenotypes in the red cherry shrimp can be described using a combination of chromatosome morphology and chroma, but the response of these pigmented units under intrinsic physiological differences and environmental constraints in general may vary. In this sense, how differences in hormonal control (Nöel, 1981; Rao, 1985) and pigment translocation and deposition influence the combined response of chromatosomes morphology and chroma in crustaceans, demands further research.

The exopod is the best body part for pigmentation monitoring in *N. denticulata* (Table 1 and Table 2). The flatness of a body part, such as uropod or telson in shrimp, has been considered as the best criterion for moulting and pigmentation observations (Abrill and Ceccaldi, 1984). In previous studies, Green (1964) found difficulties in characterizing dispersion-aggregation of chromatophores in the carapace of the fiddler crab *Uca pugnax* (Smith, 1870), opting for the observation of chromatophores in periopods, a less calcified appendage. The exopod in *N. denticulata* is weakly calcified, poorly sclerotized as well as lacking of pigments within the integument, making it more transparent and therefore suitable for epidermal chromatosome observations.

The non-invasive approach that we describe here offers the possibility to analyse colour in vivo and in different

sample sizes, as opposed to some methods which are not appropriate for small animals (Melville-Smith et al., 2003). This extends the study of pigmentation pattern in crustaceans at different stage of their life cycle and may help in our understanding of the interactions between morphology and colour in chromatosomes under different physiological and environmental scenarios.

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