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Presence of a single abundant storage hexamerin in both larvae and adults of the grasshopper, *Schistocerca americana*

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Abstract

We identified a single hexameric storage protein in the grasshopper, *Schistocerca americana*, and monitored its abundance through the last larval instar and up until reproductive competence in adults of both sexes. This storage hexamerin, termed *Schistocerca americana* Persistent Storage Protein (saPSP) was the most abundant soluble protein in both larvae and adults. In both sexes, saPSP abundance started out low at the onset of the last larval instar and accumulated during feeding, peaking just prior to molting. Adults of both sexes contained significant amounts of saPSP after eclosion. In adult males, saPSP content dropped continuously after eclosion and was lowest once individuals reached reproductive maturity. In contrast, adult females depleted saPSP reserves during the first days of adulthood, but subsequently accumulated significant saPSP stores. In adult females, saPSP stores peaked just prior to the completion of egg provisioning. Given the overall patterns of abundance, saPSP has functions in both larvae and adults. In addition, the observed pattern of storage hexamerin accumulation differs from patterns of accumulation in the other known grasshoppers, *Locusta migratoria* and *Romalea microptera*, suggesting that significant functional diversity has evolved in storage hexamerins among the grasshoppers.
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1. Introduction

Insects can store significant quantities of amino acids in storage proteins in the hemolymph and fat body. These proteins accumulate when amino acid intake is greater than demand and are used when amino acid demand exceeds intake. The ability to store amino acids has likely been essential in the evolution of several prominent insect life history traits, such as holometabolous development and reduced adult protein feeding (e.g., most lepidopterans). Several classes of proteins are known to serve storage functions, but the most common group in insects is the storage hexamerins. Insect storage hexamerins are a hemocyanin-related group of proteins composed of six identical or similar subunits in the 70–90 kDa range (Telfer and Kunkel, 1991; Burmester, 2001, 2002). Storage hexamerins occur in a wide range of species spanning the phylogeny of insects and seemingly appeared with the first hexapods (Burmester, 2002).

Storage hexamerins are synthesized by the fat body and released into the hemolymph during feeding, after which some may be sequestered back into the fat body (Telfer and Kunkel, 1991; Burmester, 2001). In holometabolous insects, these proteins accumulate in larvae and are thought to be critical in providing amino acids necessary for metamorphosis during the non-feeding pupal stage (Telfer and Kunkel, 1991; Burmester, 2001). However, more recent work in both holometabolous and hemimetabolous insects suggests additional roles for these proteins in both larvae and adults, such as providing amino acids for egg production and rebuilding tissue after adult diapause (Chinzei et al., 1990; Miura et al., 1991; Koopmanschap et al., 1992; Pan and Telfer, 1996; Seo et al., 1998; Pan and Telfer, 2001).

The functional diversity of storage proteins in insects is further evident in the variation among insect species

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in both the number of storage hexamerins employed and their amino acid compositions. For example, within the Lepidoptera, larvae of the gypsy moth *Lymantria dispar* contain a single storage hexamerin, an aromatic amino acid-rich arylphorin (Karpells et al., 1989). In contrast, larvae of the luna moth, *Actias luna*, contain three storage hexamerins, two methionine-rich hexamerins and an arylphorin (Pan and Telfer, 1996). This example illustrates that multiple classes of storage hexamerin have evolved distinct amino acid contents, presumably related to differences in their functions (e.g., the aromatic amino acid rich arylphorins and the methionine-rich hexamerins). In addition, insect species, and even life stages within a single species, can differ in the types of storage hexamerins they produce and utilize (Telfer and Kunkel, 1991; Pan and Telfer, 1992; Ancsin and Wyatt, 1996; Burmester, 2001).

A broad base of knowledge about the diversity of functions in storage hexamerins, both within and among insect species, is required to understand the evolution of this group of proteins. This knowledge has been slowly accumulating over the past three decades, however, the field has several biases. First, while storage proteins have been found in all insects so far investigated, their study has been largely focused on the Diptera and Lepidoptera (Telfer and Kunkel, 1991; Burmester, 2001). Second, because of their prominence in the last larval instar of Diptera and Lepidoptera, the functional characterization of storage proteins has focused primarily on their role in metamorphosis. There are comparatively few cases in which the role of storage hexamerins in pre-metamorphic molts or in adult functions has been studied. Unfortunately, those examples that do exist are in vastly divergent species making comparisons difficult. More studies of storage protein composition and function across the phylogeny of insects are needed, particularly in hemimetabolous insects. To this end, we identified the primary storage hexamerin occurring in the grasshopper, *Schistocerca americana* (Drury), and quantified its abundance in last instar larvae and adults through one reproductive cycle.

2. Materials and methods

2.1. Insect rearing and sampling

Insects used for this work were obtained from a *Schistocerca americana* colony maintained at The University of Arizona. This colony was founded from individuals collected near Lake Georgetown, Travis Co., Texas in October 2001. Insects were kept in a controlled environment room set at 28 °C and 20% relative humidity, and provided with 100 W light-bulbs on a 14L:10D cycle for radiant heat. Nymphs were reared in sibling groups from hatching through the penultimate (fifth) instar in 30 × 30 × 30 cm screen cages. Upon passing into the last (sixth) instar, nymphs and subsequent adults were reared individually in 800 ml plastic and screen cages until sampling. All animals were fed wheat germ and romaine lettuce ad libitum.

Male and female nymphs were sampled at three intervals during the 8–9 day-long last instar: within 12 h of molting into the last instar, 4 days into the last instar, and 8 days into the last instar. Nymphs were promptly frozen whole at −70 °C. Adult females were sampled every four days between eclosion and just prior to egg laying, which occurs approximately 20 days into adulthood (Days 0, 4, 8, 12, 16, and 20). At the time of sampling, adult females were anesthetized, their ovaries dissected out, and carcasses were frozen at −70 °C. As a measure of reproductive status, egg development was observed in dissected ovaries, and the length of developing eggs was measured to the nearest 0.01 mm using a microscope-mounted optical micrometer. Adult males were sampled every five days for the first 20 days of life as they became reproductively mature (Days 0, 5, 10, 15, and 20). Some males are competent to mate within 15 days after eclosion, and all are competent by 20 days (D. Hahn, unpublished work). Males were immediately frozen whole at −70 °C. After freezing, all individuals were freeze-dried until they reached constant mass, and weighed. Dry masses were used to estimate somatic growth. All individuals were subsequently homogenized in liquid nitrogen and stored at −70 °C until analysis.

2.2. Sample preparation and electrophoresis

Samples were prepared for electrophoresis by agitating a known quantity of homogenate from each individual in 0.3 ml of Tris-buffered saline (20 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 7.5) containing the following protease inhibitors: leupeptin, antipain, chymostatin, aprotinin (all at 17 μg/ml), 1.7 μg/ml pepstatin A, and 1 mM 4-(2-Aminoethyl) benzenesulfonyl Fluoride (AEBSF, an irreversible serine protease inhibitor). Samples were agitated in 1.5 ml plastic microcentrifuge tubes with a plastic pestle attached to a rotating shaft driven by a variable speed motor. Each sample was ground for 60–90 s at 250 rpm, then centrifuged at 12,000 for 20 min at 4 °C. Soluble proteins remained in the supernatant.

Storage protein content differed greatly among individuals across the life stages sampled. Consequently, the supernatant for each sample was diluted using the above tris-buffered saline protease-inhibitor solution as needed to bring it into quantifiable range. Ten μl aliquots were taken from the appropriate dilution, mixed with 20 μl of sample loading buffer, and loaded onto gels.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed following

Laemmli (1970), adapted to 6–15% gradient slab gels. Gels were run at 20 mA constant current and stained with 0.1% Coomassie Brilliant Blue R 250 dissolved in a 5:4:1 solution containing methanol, water, and acetic acid. Gels were destained in the same solution without Coomassie. Molecular weights of apoproteins were estimated in SDS-PAGE using standards in the high molecular weight calibration kit (Bio-Rad) containing proteins with the following molecular weights: myosin (200,000 kDa), galactosidase (116,250 kDa), phosphorylase B (97,400 kDa), BSA (66,200 kDa), and ovalbumin (45,000 kDa).

To assess proteins in their native state, 4–20% native-PAGE gradient gels were used. The buffer system of Laemmli (1970) was used, with SDS and beta-mercaptoethanol omitted. Molecular weights of native proteins were estimated using the following standards obtained from Pharmacia: thyroglobulin (669,000 kDa), ferritin (440,000), catalase (232,000 kDa), lactate dehydrogenase (140,000 kDa), and albumin (67,000 kDa).

2.3. Amino acid composition and N-terminal sequencing

Purification of storage proteins was performed using 4–20% native-PAGE gradient gels. Proteins were electrophoretically transferred to a PVDF (polyvinylidene difluoride) membrane (Problott®). After staining with 0.1% Coomassie brilliant blue R 250, bands of interest were cut out. Amino acid analysis was performed at The University of Arizona Biotechnology Core Facility using a dedicated Applied Biosystems Model 420A Amino Acid Analyzer with automatic hydrolysis (vapor phase at 160 °C for 100 min using 6 M HCl) and pre-column phenylthiocarbamyl-derivatization. The amino acid composition of the primary storage hexamerin (*Schistocerca americana* Persistent Storage Protein, saPSP) was analyzed from two independent samples purified from female larvae and two independent samples purified from female adults. The mean percent compositions for each amino acid and one standard error for all four runs are presented in Table 1. Protein sequencing was performed at the same facility using an Applied Biosystems 477A Protein/Peptide Sequencer interfaced with a 120A HPLC for on-line phenylthiohydantoin amino acid analysis. Alignment and similarity between the *S. americana* PSP sequence and the *L. migratoria* PSP sequence (Ancsin and Wyatt, 1996) was assessed using CLUSTALW.

2.3.1. Statistical analyses of amino acid composition

We predicted that, if saPSP is a storage hexamerin, it would contain a similar percentage of aromatic amino acids to the known *L. migratoria* storage hexamerins and a greater percentage than a proxy for the average insect protein calculated from the Fly Base data-base (http://www.flybase.org). One-way ANOVA was used to compare the mean aromatic amino acid content of saPSP, the known *Locusta migratoria* storage hexamerins, and the average insect protein estimate from Fly Base. Groups within the ANOVA were compared using an a posterori Tukey's HSD test for multiple comparisons. Aromatic amino acid content was calculated as the mole percent phenylalanine content plus the mole percent tyrosine content for each protein. The values for the *L. migratoria* storage hexamerins were taken from de Kort and Koopmanschap (1987) and Ancsin and Wyatt (1996). To generate an estimate for the average insect protein, amino acid sequences for 50 *Drosophila melanogaster* proteins were obtained from Fly Base. These proteins were selected on the basis of gene functions in biological processes as described by Gene Ontology (http://www.geneontology.org) in a manner that was consistent with the number of genes in each gene ontology functional group identified in the *D. melanogaster* genome (Adams et al., 2000). Some function subcategories were too small to be included in a representative sample of 50 proteins. The FlyBase gene numbers of the proteins used were: 0000097, 0000229, 0000313, 0000340, 0000497, 0000536, 0000556, 0000557, 0000635, 0000636, 0001075, 0002716, 0003285, 0003300, 0003301, 0003391, 0003502, 0003411, 0003714, 0004170, 0004864, 0004956, 0005775, 0010109, 0010341, 0011762, 0014075, 0015582, 0015609, 0016036, 0016131, 0016917, 0016920, 0020392, 0024732, 0024836, 0027363, 0028406, 0028737, 0029176, 0031392, 0032117, 0032198, 0032655, 0036485, 0036930, 0041184, and 0044872. This proxy agrees well with two other estimates of average aromatic amino acid composition across many taxa, one compiled by King and Jukes (1969) for 53 vertebrate proteins and one for thousands of proteins from a variety of organisms provided by the Swiss-Prot Database (us.expasy.org/tools/pscale/A.A. Swiss-Prot.html). Therefore, we concluded that the average aromatic amino acid content calculated from FlyBase is an appropriate proxy for the average insect protein.

2.4. Western blotting

G.R. Wyatt generously provided antibodies to two known *L. migratoria* storage hexamerins, one specific to larvae (lmLSP-1), and one that occurs in both larvae and adults (lmPSP) (Ancsin and Wyatt, 1996). *S. americana* nymph and adult proteins were prepared as above, separated on a 4–20% native-PAGE gel, and blotted onto nitrocellulose paper. After blotting, the paper was rinsed thoroughly, air-dried, and stored at −20 °C. Blots were probed with a 1:1000 dilution of the primary antibody, incubated with a 1:3000 dilution of goat anti-rabbit secondary antibody, conjugated with alkaline phosphotase and detected with BCIP NBT color reagent (Bio-Rad).

Table 1
Amino acid composition of the *Schistocerca americana* storage hexamerin compared with values for three known *Locusta migratoria* storage hexamerins, the average of the proteins in the Swiss-Prot database, King-Jukes averages, and the averages for 50 *Drosophila melanogaster* protein sequences from FlyBase

Amino acid (mol%)	*Schistocerca americana*	*Locusta migratoria*			DM 50 average protein	Swiss-Prot	King-Jukes
	PSP[a]	PSP[b]	LSP-1[b]	LHP[c]			
Asparagine/aspartic acid	9.13 ± 0.17	8.8	8.1	10.1	10.7	9.6	10.3
Glutamine/glutamic acid	12.33 ± 1.2	11.7	10.9	10.2	10.9	10.4	9.5
Serine	6.05 ± 0.44	6.5	4.5	4.5	8.2	7.1	8.1
Glycine	10.1 ± 1.38	8.6	5.3	6.9	6.0	6.9	7.4
Histidine	1.25 ± 0.19	4.2	2.0	1.2	2.6	2.2	2.9
Arginine	4.03 ± 0.34	6.0	10.8	7.0	5.6	5.2	4.2
Threonine	3.13 ± 0.22	4.5	3.3	4.0	5.7	5.6	6.2
Alanine	7.25 ± 0.21	9.9	8.0	8.4	7.2	7.6	7.4
Proline	6.73 ± 0.46	4.9	6.4	6.2	5.5	4.8	5
Lysine	4.5 ± 0.24	5.5	3.9	6.1	5.6	6.0	7.2
Valine	7.33 ± 0.38	7.4	6.6	7.4	6.5	6.6	6.8
Isoleucine	4.75 ± 0.24	3.7	5.2	5.6	5.0	5.9	3.8
Leucine	11.28 ± 0.5	8.4	9.0	9.0	9.1	9.5	7.6
Phenylalanine	6.58 ± 0.48	4.9	5.4	5.5	3.4	4.1	4.3
Tyrosine	5.42 ± 0.46	4.1	8.3	6.8	3.2	3.2	3.3
Total aromatics	11.95 ± 0.95	9.0	13.7	12.3	6.6	7.3	7.6

[a] Data are the mean and one standard error for four different analyses of this protein.
[b] Data taken from Ancsin and Wyatt (1996).
[c] Data taken from de Kort and Koopmanschap (1987).

2.5. Protein quantification

Because *S. americana* PSP disassociated under native-PAGE conditions, SDS-PAGE was used to quantify storage hexamerin apoproteins present in various stages of development. Sub-units of two other proteins (a putative JH-binding protein, and a putative lipophorin) co-migrated with the apoproteins of saPSP on SDS-PAGE gels and were quantified together. Because saPSP was the most abundant soluble protein in both nymphs and adults, always occurring in much greater concentration than these other proteins, SDS-PAGE gels provided an adequate estimate of saPSP abundance. SDS-PAGE gels were run and stained with Coomassie as described above. To quantify storage proteins, gels were scanned at 633 nm using a laser densitometer (LKB Ultrascan XL). Standard curves were generated using known quantities of bovine serum albumin (BSA) that ranged from 0.2 to 4.0 μg. Internal standards of 1.0 and 3.0 μg BSA were included on each gel to correct for gel to gel variation.

2.5.1. Statistical analysis of storage protein content, dry mass, and egg development

Storage protein accumulation was quantified during the last instar in larvae and until reproductive age in adults of both sexes. Accumulation in larvae was analyzed using multiple factor ANOVAs with age, sex and dry mass as effects in the models. Differences in accumulation of storage protein, dry mass and egg development between male and female adults were expected a priori. Therefore, adults of each sex were analyzed separately. Differences among age groups within each sex were assessed using one-way ANOVA, followed by an a posterori Tukey's HSD correction for multiple comparisons.

3. Results

3.1. Molecular weights and antibody recognition

A single storage hexamerin was the most abundant soluble protein in both nymphs and adults (Fig. 1a and b). Like the Persistent Storage Protein described by Ancsin and Wyatt (1996) in the grasshopper, *Locusta migratoria* (lmPSP), this hexamerin disassociated in native-PAGE, was present in both larvae and adults, and showed significant cross-reactivity with an antibody raised to lmPSP (Fig. 2). Henceforth, this protein is referred to as *Schistocerca americana* Persistent Storage Protein (saPSP). SaPSP had an estimated native molecular weight of 470 kDa with apoproteins of approximately 72 kDa (Fig. 1a and b). Like lmPSP and some other storage hexamerins, the bulk of saPSP disassociated into smaller quaternary structures consisting primarily of tetramers (approximately 290 kDa), dimers (approximately 140 kDa), and monomers when run on native-PAGE gradient gels (Fig. 1a).

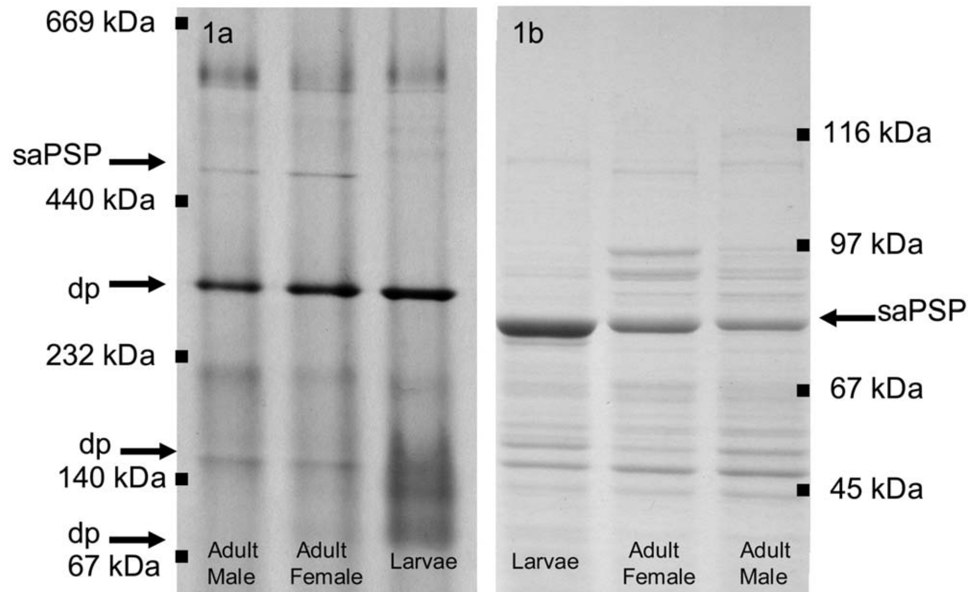

Fig. 1. (a) Four to twenty percent native-PAGE gradient gel showing the most abundant storage hexamerin (saPSP⟶) and the products of its disassociation (dp⟶) in nymphs, adult males, and adult females. (b) Six to fifteen percent SDS-PAGE gradient gel showing apoproteins of saPSP in nymphs, adult males, and adult females (saPSP⟶). Molecular weights for native-PAGE are listed on the left and those for SDS-PAGE on the right (black squares).

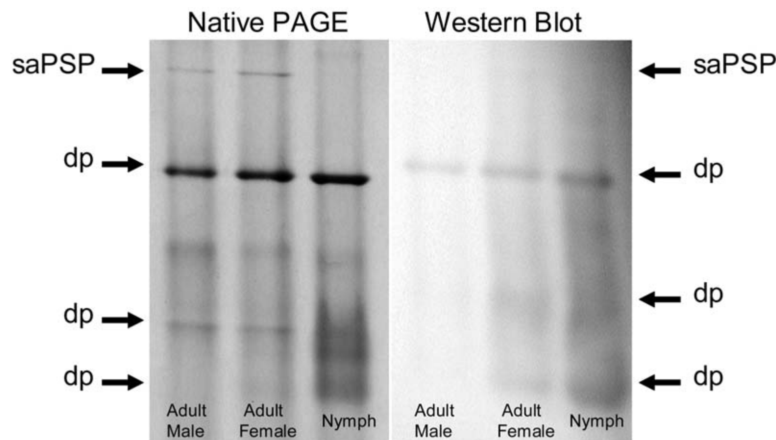

Fig. 2. Four to twenty percent native PAGE-gradient gel (left) and western blot (right) of larval, adult female, and adult male samples exposed to antiserum raised to the Persistent Storage Protein of *Locusta migratoria* (Ancsin and Wyatt, 1996).

3.2. Amino acid composition and N-terminal sequence

The mean mol% amino acid composition and one standard error for the four saPSP amino acid analyses are listed in Table 1. Because insect storage hexamerins typically contain higher than average aromatic amino acid compositions, we compared the mean aromatic amino acid content, defined as mol% tyrosine plus mol% phenylalanine, for saPSP to the three known *Locusta migratoria* storage hexamerin estimates and an estimate of the average *Drosophila melanogaster* protein determined from 50 protein sequences obtained from Fly Base. As expected, both saPSP and the *L. migratoria* storage hexamerins contained significantly greater aromatic amino acid content than the average *D. melanogaster* protein (means: 95% CI, saPSP = 12.00:10.04–13.96, LM = 11.67:9.41–13.93, DM = 6.63:6.07–7.18, ANOVA, $F_{2,56} = 22.05$, $p < 0.001$, Tukey's HSD, $p < 0.05$ for both). There was no difference in aromatic amino acid content between saPSP and the *L. migratoria* storage hexamerins (Tukey's HSD, $p > 0.05$). The N-terminal sequences of *S. americana* PSP and *L. migratoria* PSP were identical at 19 of 20 residues (Fig. 3), indicating homology between these two proteins.

3.3. Patterns of accumulation in larvae

For larvae of both sexes, storage protein content was low at the beginning of the last instar, increased through

																	*			
Schistocerca americana	A	V	V	P	H	S	E	A	G	K	E	L	L	E	K	Q	V	K	L	L
Locusta migratoria	A	V	V	P	H	S	E	A	G	K	E	L	L	E	K	Q	E	K	L	L

Fig. 3. Alignment of the N-terminal amino acid sequences from *S. americana* PSP and *L. migratoria* PSP (Ancsin and Wyatt, 1996). The two sequences are identical at 19 of 20 amino acids, * denotes the only difference between the two sequences.

the instar, peaked just prior to molting and dropped again in newly eclosed adults (Fig. 4a). Storage protein content was slightly, but not significantly higher in female nymphs throughout the last instar and after adult eclosion (Fig. 4a and Table 2a). However, female nymphs gained more mass than male nymphs during the last instar as evidenced by the significant age × sex interaction term in the ANOVA (Fig. 4b and Table 2b). When mass was added into the statistical model, neither age nor sex had a significant effect on storage protein content (Table 2c).

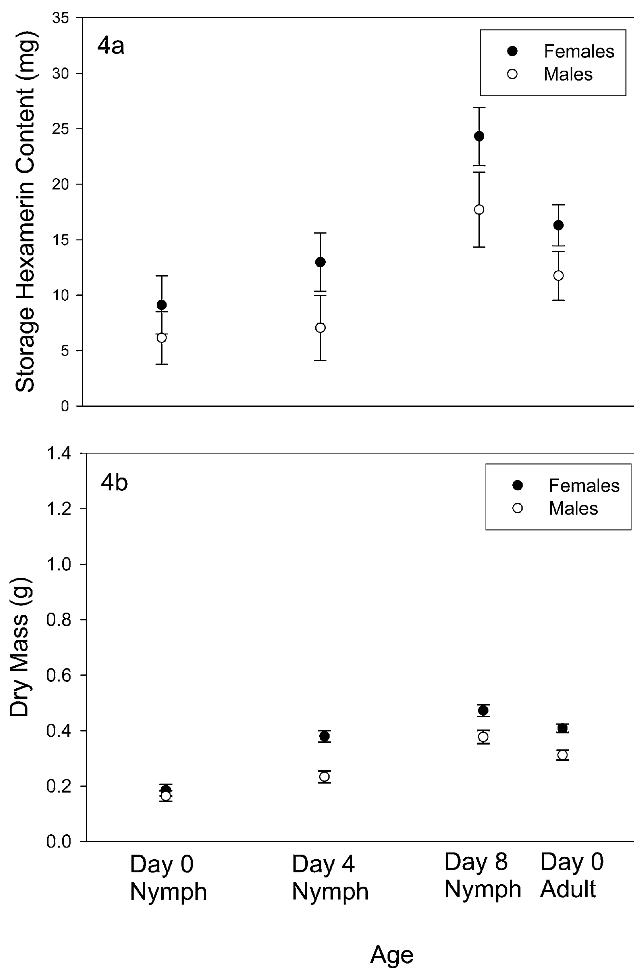

Fig. 4. (a) Storage hexamerin content of male (gray circles) and female (black squares) nymphs, and newly eclosed adults. (b) Dry mass of male (gray circles) and female (black squares) nymphs, and newly eclosed adults. Horizontal bars represent one standard error.

Table 2
ANOVA tables for effects on dry mass and storage protein content in nymphs

Source	df	*F*	*p*
(a) *Effects of age and sex on storage protein content in nymphs*			
Whole model	4	10.16	<0.001
Age	3	9.70	<0.001
Sex	1	7.81	0.008
Error	40		
Total	44		
(b) *Effects of age and sex on dry mass in nymphs*			
Whole model	7	32.38	<0.001
Age	3	53.85	<0.001
Sex	1	0.55	0.464
Age × sex	3	3.18	0.034
Error	39		
Total	46		
(c) *Effects of age, sex, and dry mass on storage protein content in nymph*			
Whole model	5	11.48	<0.001
Age	3	2.16	0.108
Sex	1	0.08	0.780
Dry mass	1	8.80	0.005
Error	39		
Total	44		

3.4. Patterns of accumulation in adult males

In males, storage protein content was highest just after eclosion and declined throughout adulthood (one-way ANOVA, $F_{4,28} = 3.48$, $p = 0.022$, Fig. 5a). Significant somatic growth occurred in males after eclosion, with mass plateauing after 10 days of adulthood (one-way ANOVA, $F_{4,28} = 12.82$, $p < 0.001$, Fig. 5b).

3.5. Patterns of accumulation in adult females

As in adult males, storage protein content declined after eclosion in adult females (Fig. 6a). However, in contrast to adult males, storage protein content rose again in adult females after the first week, peaked during the time of maximum egg provisioning, and dropped off slightly as eggs reached maturation (one-way ANOVA, $F_{5,56} = 3.51$, $p = 0.008$). Egg provisioning began approximately 10 days into adulthood and progressed until 20 days of adulthood when all eggs were mature and ready to be laid (one-way ANOVA, $F_{5,61} = 24.25$, $p < 0.001$, Fig. 6b). Total somatic dry mass of females,

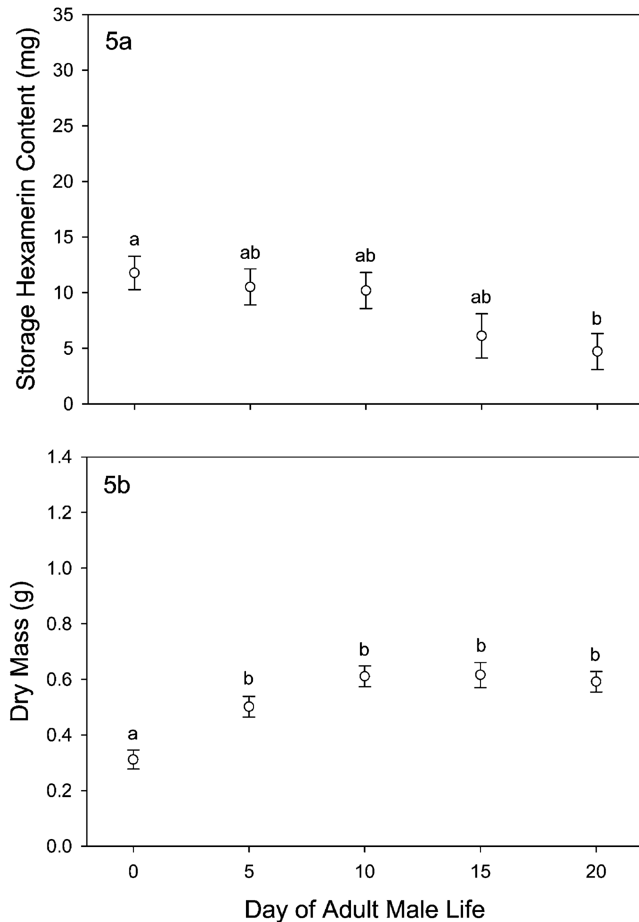

Fig. 5. (a) Storage hexamerin content of adult males until the time of reproductive competency. (b) Dry mass of adult males until the time of reproductive competency. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ($\alpha = 0.05$).

not including ovaries and developing eggs, steadily increased from eclosion until egg provisioning was completed (one-way ANOVA, $F_{5,56} = 26.17$, $p < 0.001$, Fig. 6c).

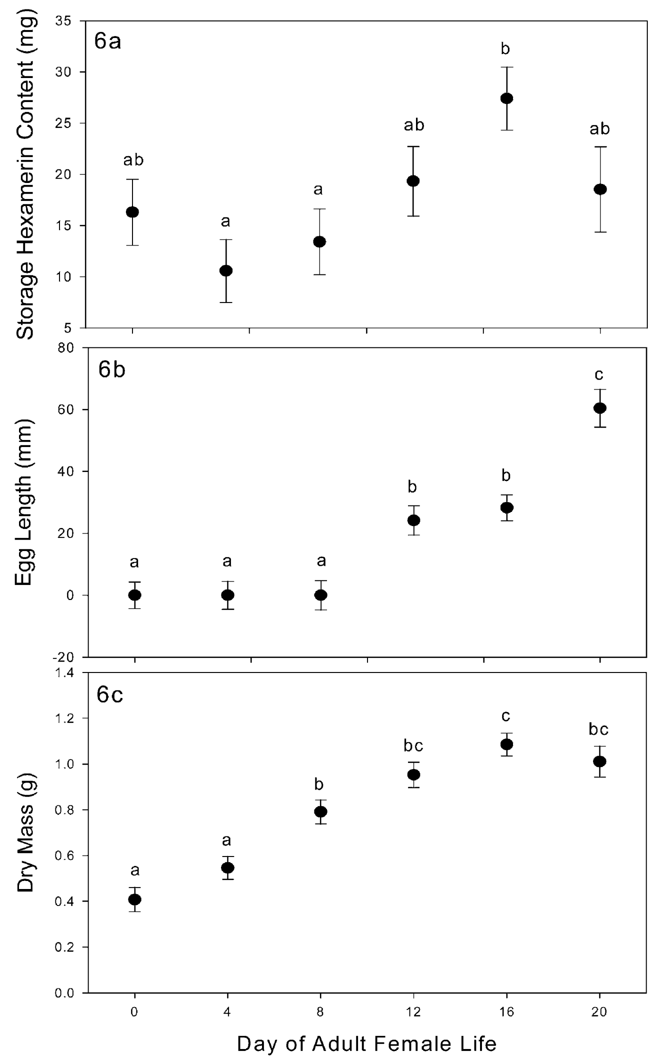

Fig. 6. (a) Storage hexamerin content of adult females until the time of egg laying. (b) Development of eggs (egg length) in females until the time of laying. (c) Dry mass of adult females until the time of egg laying. Horizontal bars represent one standard error. The same letter denotes groups that are not significantly different after a Tukey's HSD correction for multiple comparisons ($\alpha = 0.05$).

4. Discussion

We identified a single abundant storage hexamerin (saPSP) in both larvae and adults of both sexes of the grasshopper, *Schistocerca americana* that is homologous to the persistent storage protein described in larvae and adults of the African Migratory Locust, *Locusta migratoria* (lmPSP) (Ancsin and Wyatt, 1996).

As in other insects, this storage hexamerin accumulated during larval feeding, with female larvae, which are larger, accumulating slightly greater amounts than male larvae, peaked just prior to molting, and was partially depleted during molting to adulthood. Individuals of both sexes contained significant saPSP reserves just after adult eclosion, but these reserves were largely depleted in the first few days of adulthood, a period of rapid somatic growth. The disappearance of post-eclosion protein stores suggests that saPSP accumulated during larval feeding is used to construct adult tissues both at the time of molting and after eclosion when rapid somatic growth occurs in both sexes. Because adults do not begin feeding until 12–24 h after eclosion, storage hexamerins present just after eclosion must either be carried over from larval life or synthesized from surplus amino acids not used during metamorphosis. However, within 36 h of eclosion all adults begin feeding. Therefore, amino acids available for use in the period of rapid somatic growth that occurs during the first week of adulthood can be derived from either larvally accumulated reserves or adult feeding. To understand the function of saPSP in adult growth, it will be necessary to

determine its contribution to both growth during molting and post-eclosion somatic growth.

In adults, patterns of storage hexamerin content differed between the sexes. In males, saPSP content declined during adulthood, reaching its lowest level just prior to reproduction when sperm and accessory gland proteins are produced. In addition to producing sperm, many male orthopterans invest significant amounts of amino acids in constructing a protein-rich spermatophore (Uvarov, 1966). Therefore, *S. americana* males may require considerable amounts of amino acids to support reproduction. In contrast, while females also showed slight depletion of saPSP early in adulthood, females accumulated considerable amounts of saPSP prior to egg laying, with the peak occurring just prior to egg maturation. This pattern of storage hexamerin cycling with egg provisioning suggests that females are using saPSP as an intermediate amino acid store for provisioning eggs. Storage proteins are known to be used in egg provisioning in other insects, so it seems likely that *S. americana* uses them in this capacity as well (Chinzei et al., 1990; Pan and Telfer, 1996; Seo et al., 1998; Pan and Telfer, 2001). In addition, at the time of egg laying, *S. americana* females require significant amounts of amino acids to produce a protein-rich egg casing and foam plug which covers the egg pod and extends to the soil surface several centimeters above (Uvarov, 1977). To understand the function of saPSP in *S. americana* adults, it will be necessary to document whether amino acids from this protein are used for gamete or accessory protein production in either sex.

Interestingly, the pattern of hexamerin accumulation in the American Locust, *S. americana*, differs from that observed in two other well-studied orthopteroid insects; the Migratory Locust, *Locusta migratoria* and the German Cockroach, *Blatella germanica*. In *L. migratoria*, two storage hexamerins are found only in larvae (lmLSP-1 and lmLSP-2), and a third is found in both larvae and adults (lmPSP) (Ancsin and Wyatt, 1996). In *L. migratoria* larvae, lmLSP-1 occurs in the highest concentration, followed by lmPSP, with lmLSP-2 only occurring in very low concentrations. In *L. migratoria* adults, lmPSP is the only detectable storage hexamerin, where it can occur in concentrations as high as those observed for lmLSP-1 in larvae. The case is similar for *B. germanica* where larvae contain two identified storage hexamerins (bgLSP and bgPSP), but only one remains in adults (bgPSP) (Duhamel and Kunkel, 1978; Telfer and Kunkel, 1991). In *B. germanica* larvae, bgLSP occurs in much higher concentrations than bgPSP, but in adults only bgPSP is detectable. In both these cases, one protein occurs in much higher concentrations in larvae and the other in much higher concentrations in adults, leading these authors to suggest that these proteins may be specialized for larval and adult functions, respectively.

In contrast to the above examples, a single storage hexamerin (saPSP) is the most abundant soluble protein in both larvae and adults of *S. americana*. This suggests that saPSP serves significant functions in both life stages. It is possible that *S. americana* contains other storage hexamerins, but they were not detectable using the methods in this study. Regardless of whether other storage hexamerins occur in *S. americana*, the fact that a single storage hexamerin occurs in large quantities in both larvae and adults demonstrates a clearly different pattern from the other described grasshopper and cockroach. In addition, recent work shows that the lubber grasshopper, *Romalea microptera*, contains three putative storage hexamerins, all three of which occur in significant quantities in both larvae and adults (Hatle et al., 2001, J.D. Hatle personal communication). Therefore, studies of the storage hexamerins in three species of short-horned grasshoppers (Acrididae) show three different patterns of accumulation and depletion. Because all three species represent different subfamilies and have somewhat different life histories, perhaps it is not surprising that there are differences in their biochemistry and physiology. However, two questions are essential to understanding the evolution of differences in storage protein accumulation among these three grasshopper species. First, do the three species differ in the number or structure of storage hexamerin genes they contain, or just in their expression and product accumulation? Second, how do the functions of each protein differ in nymphs and adults of each species? Clearly, more comparisons of storage hexamerin identity and function are needed across insects, especially within the hemimetabola, to understand the evolution of insect storage hexamerins. We encourage others to document the presence, accumulation patterns, and functions of storage hexamerins in a variety of taxa as a first step towards understanding the diversification of functions and expression patterns of insect storage hexamerins.

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