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Comparison of life history and genetic properties of cowpea bruchid strains and their response to hypoxia

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ABSTRACT

The cowpea bruchid (*Callosobruchus maculatus*) is the most important storage pest of grain legumes and comprises geographically distinct strains. Storage under a modified atmosphere with decreased O_2 content represents an alternative to chemical fumigants for pest control of stored grains. In this study, we compared reproduction, development and survival, as well as genome size of bruchid strains from South India (SI), Burkina Faso (BF), Niger (CmNnC) and the United States (OH), reared on mung bean (*Vigna radiata*). Fecundity and egg-to-adult duration varied significantly among these strains. Notably, strain BF had the highest fecundity, and strain SI displayed the fastest development whereas strain OH was the slowest. Differences in adult lifespan among strains were only detected in unmated but not in the mated group. Genome size of SI females was significantly larger than that of OH females, and for all four strains, the female genomes were larger than those of their corresponding males. Furthermore, we studied effects of exposure to 1% O_2 + 99% N_2 on strains SI and BF. Mortality caused by hypoxia was influenced by not only developmental stage but also by insect strain. Eggs were most sensitive, particularly at the early stage, whereas the 3rd and 4th instar larvae were most tolerant and could survive up to 15 days of low O_2. Strain SI was slightly more resistant than BF in egg and larval stages. Proteolytic activity prior to, during and after hypoxia treatment revealed remarkable metabolic plasticity of cowpea bruchids in response to modified atmosphere.

1. Introduction

Terrestrial insects are dependent on atmospheric O_2 for generation of metabolic energy. Disinfestation of storage pests using a modified atmosphere with low O_2 (hypoxia) and/or high CO_2 (hypercapnia) content represents an alternative to fumigation with synthetic insecticides such as methyl bromide (Fields and White, 2002; Fleurat-Lessard, 1990). A hypoxic and/or hypercapnic environment can be achieved by hermetically sealing storage units so that the O_2 consumed by infesting insects in the storage units cannot be replaced, while respiration causes increased CO_2 concentration (Murdock et al., 2003; Sanon et al., 2011). Other approaches include directly purging the storage facility of air (O_2) using N_2 or CO_2, or by introducing gases generated outside the storage container from combustion of hydrocarbon fuels (Conyers and Bell, 2007). Depending on gas composition, exposure time, insect species and their developmental stages, effects of hypoxia vary (Donahaye et al., 1996; Hoback and Stanley, 2001; Soderstrom et al., 1990; Wang et al., 2001). Considerable research has been conducted on cosmopolitan pests such as *Tribolium castaneum*, *Tribolium confusum*, *Sitophilus zeamais*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus* and *Oryzaephilus surinamensis* (Carli et al., 2010; Chiappini et al., 2009; Finkelman et al., 2006; Lord, 2009; Riudavets et al., 2009). Generally, a reduction in the O_2 content to 3% or lower or an increase in CO_2 content to 60% or higher is effective for control of most storage pests (Navarro, 2006).

The cowpea bruchid, *Callosobruchus maculatus* (Coleoptera: Bruchidae) is the primary pest of stored legume seeds, especially the cowpea, *Vigna unguiculata* Walp. This bruchid thrives wherever its hosts are grown and stored, particularly in Africa where grain legumes are essential protein sources (Langyintuo et al., 2003). Infestations by the bruchids begin in the fields, but populations expand rapidly in storage. Adult females deposit their eggs on the seed surface, and hatched larvae burrow into and feed inside the seeds, where they complete four-instar larval and pupal

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development. Upon emergence, adults begin to mate and oviposit within a few hours, initiating another round of infestation. Complete infestation of cowpea can occur after 3–5 months of storage (Ajayi and Wintola, 2006). Damaged seeds can be completely hollowed out by feeding larvae, causing a severe loss of seed weight, nutrition, germination potential, and thereby the commercial value of the commodity (Boeke et al., 2004).

Geographically different populations of cowpea bruchids, defined as strains, vary in numerous biological parameters. For instance, fecundity, developmental period, mortality and sex ratio differ significantly among bruchid strains Campinas, Yemen and IITA (Dick and Credland, 1984). The male's ejaculate size during mating and female's egg-spacing behavior are highly variable among populations collected worldwide (Messina and Mitchell, 1989; Savalli et al., 2000). Differences in larval respiration rate and seed consumption were also detected among strains (Guedes et al., 2003). Many of these variations are genetically-based (Bieri and Kawecki, 2003; Kawecki, 1995; Messina and Slade, 1997). Despite considerable investigation of bruchid strains, not all populations are well documented. For example, a strain originally collected from Columbus, Ohio (strain OH) is among the undocumented. Furthermore, genome size variation is thought to contribute to life history variation (Biemont, 2008; Ellis et al., 2014; Hessen et al., 2013), yet no information is available on genome size of bruchid strains so far. In addition, although hypoxia is known to affect growth and development of cowpea bruchids (Cheng et al., 2012, 2013; Mbata et al., 1996; Ofuya and Reichmuth, 1993; Storey, 1978), it is unclear whether hypoxia has differential impacts on various bruchid strains.

In this study, we compared biological (reproduction, development and mortality) and genetic (genome size) parameters of four different cowpea bruchid strains originating from Africa, Asia and America. We then investigated the hypoxic responses of two of these strains by comparing mortality at different developmental stages of two strains when exposed to 1% O_2 + 99% N_2, and measured their midgut proteolytic activities under hypoxia and normoxia.

2. Materials and methods

2.1. C. maculatus strains

The four cowpea bruchid strains used in the current study were collected originally from infested cowpeas in Niamey, Niger (CmNnC) and Ouagadougou, Burkina Faso (BF), and from infested mung beans in Tirunelveli, South India (SI) and Columbus, Ohio, the United States (OH), respectively. Prior to this study, strain SI had been maintained on mung bean seeds, whereas strains CmNnC, BF and OH had been maintained on cowpea seeds for over 10 generations. Comparisons of life history traits, genome sizes and hypoxic response among bruchid strains were performed on mung bean. To minimize possible previous host effects, new populations of strains CmNnC, BF and OH (previously maintained on cowpea) were grown for two generations on mung bean before the start of these experiments. All cultures of four strains were maintained in 500 mL wide-mouth glass bottles, and all experiments in this study were conducted in an environmental chamber with 27 °C and 60% R.H.

2.2. Reproduction, development and mortality of cowpea bruchid strains

For each bruchid strain, approximately 200, 1–4 day old adults were introduced into a wide-mouth glass bottle containing 400 mung beans for mating and oviposition. The adults were removed 2 h later to obtain an age-synchronized population. Seeds with a single egg on a mung bean were selected. Batches of 50 eggs (4–6 h old) were placed into 30 mL clear plastic cups with their lids and sides perforated for air exchange. The number of hatched eggs was recorded 8 days later, using color change as the indicator of hatching (Shazali et al., 2004). Since larvae were hidden inside the seeds, the number of successfully hatched eggs was used to determine the initial larval and pupal numbers in the seeds. Emerged adults from these seeds were recorded twice a day until no further emergence occurred. There were three replicates for each strain. Egg viability, adult emergence from hatched eggs, and egg-to-adult developmental duration were calculated.

To determine the longevity of mated adults and the fecundity of individual females of four strains, 25 mung beans were introduced into 30 mL clear perforated plastic cups. Three 0–4 h old virgin adults (one female and two males) of a single strain were released into each cup, and deaths of female and male adults were recorded daily. The total number of eggs laid by each female was also documented. This was repeated ten times for each strain. To measure longevity of unmated adults, 5 females or males of each strain, 0–4 h old, were released into a 30 mL perforated clear plastic cup for observation of adult death. This experiment was repeated six times for each bruchid strain.

2.3. Measurement of genome sizes of cowpea bruchid strains

Male and female adults from each of the four strains were collected and prepared for genome size estimates as described in Hare and Johnston (2011). Briefly, the head of a single male or female adult was placed into 1 mL ice-cold Galbraith buffer in a 2 mL Dounce tissue grinder along with the head of a single female *Drosophila virilis* which served as a genome size standard (1C = 328 Mb). The heads were ground and the resultant solution filtered through 40 μm nylon mesh and then stained in 25 μg/mL propidium iodide for 30 min. The relative fluorescence of diploid nuclei from the head of the bruchid and the standard were scored using a CyFlow flow cytometer (Partek America, Swedesboro, NJ). The 1C amount of DNA in each bruchid was calculated as the ratio of the mean 2C fluorescence of the bruchid and standard times the amount of DNA in the standard. A minimum of 9 adults for each sex of each strain were scored.

2.4. Hypoxic treatment of strains BF and SI

Due to their similar developmental time, strains BF and SI were subjected to 1% O_2 + 99% N_2 treatment to determine whether low O_2 differentially influenced mortality of different bruchid strains. The precise developmental sub-stages for both strains (Table 1) were pre-determined as previously described (Cheng et al., 2012), and prepared accordingly. Seeds, each carrying a single insect at a specified developmental stage including eggs of three stages (early, intermediate and black-headed), or larvae of four instars or pupae were selected.

Certified pre-mixed gas (1% O_2 + 99% N_2) was purchased in the form of pressurized cylinders from Brazos Valley Welding Supply (Bryan, TX). Batches of (respectively) 50 eggs of each stage, 30 larvae of each instar, 30 pupae and 20 adults (ten females and ten males, 4–6 h old) were separately placed into one-liter septum bottles (Industrial Glassware, Millville, NJ) and exposed to 1% O_2 + 99% N_2. Specifically, the septum bottles with infested mung bean seeds were connected to the outlet of the pressure gage of gas cylinders via plastic tubing. The gas was delivered to the bottles for 7 s at 70 kPa as measured on a manometer and controlled by a regulator. After treatment, the bottles were immediately sealed to prevent any diffusion of air. The level of O_2 was verified using a head-space analyzer (Mocon PAC CHECK®, Model 325, Minneapolis, MN). The