devised by POLITOFF et al.[12] in which its glutamine is replaced by tryptophan[2]. As shown elsewhere[2], this medium induces puffs at the following loci: Ia3/4, Ie3, If4, Ig1, Ig2, IIId1 and IIId1/2.

At 100 µg/ml cordycepin, offered in hemolymph or Ringer solution, caused in 80–85% of the cells (n = ca. 1000) a nearly total inhibition of ^{3}H-uridine incorporation into nucleoli and chromosomes (Figure 1), a general regression of all puffs and a collapse of the Balbiani rings (Figure 2). No new puffs were induced by cordycepin. At lower concentrations (10, 25, 50 µg/ml) neither inhibition of ^{3}H-uridine incorporation nor puff regression was detectable.

In accordance with previous findings[8, 2], exposition of salivary glands to test media I and II gave rise to the induction of the puffs listed above in about 70% (TM I) and 80% (TM II) of the cells. The puffs thus induced incorporate ^{3}H-uridine.

Inclusion of 100 µg/ml cordycepin in TMI or TM II blocked the induction of any of the puffs listed above in 80–85% of the cells (Figure 3). In 15–20% of the cells these puffs did appear at a size approximately equal to that of the controls (n = ca. 1000).

Discussion. The observation that isolated polytene chromosomes incubated in simple salt solutions exhibit a differential decondensation of bands ('swelling' and 'fading')[3] in conjunction with the results reported in this communication, points to a 2 step mechanism in the formation of puffs: a) an initial, highly localized, decondensation of DNP fibrils, which is independant of energy and RNA synthesis and b) a subsequent step which involves further decondensation, engulfing of neighbouring bands and an accumulation of acidic proteins[13]. This second step, which creates the 'puff-structure', seems to require energy[2] and RNA synthesis[6, 14]. It appears that ions are capable of effecting both steps.

That 15–20% of the cells show 'resistance' to cordycepin demonstrates a heterogenity in the cell population of salivary glands. Such a heterogeneity was also observed by BEERMANN[14] in *C. tentans* salivary gland cells in their reaction to α-amanitin. It is possible that the 'resistant cells' are the same which were found by KROEGER et al.[15] to have an 'inverted' Na/K ratio and which they assumed to be approaching DNA synthesis or to be engaged in it[16].

Cordycepin seems to block RNA synthesis by preventing the addition of poly(A) residues at the 3'-terminus of the HnRNA[17]. Since other inhibitors of RNA synthesis like actinomycin D[6] and α-amanitin[14] also inhibit puff formation, it is probable that the effects of cordycepin reported in this paper are due to inhibition of RNA synthesis rather than any other specific facet of its action[18].

Zusammenfassung. In 80–85% aller Speicheldrüsenzellen von *Chironomus thummi* bewirkt 100 µg/ml Cordycepin: 1. Fast vollständige Hemmung des ^{3}H-Uridin-Einbaues in Chromosomen und Nukleolen. 2. Rückbildung aller vorhandenen Puffs und Kollaps der Balbiani-Ringe und 3. Hemmung der Puff-Induktion durch anorganische (K/Mg) und organische (Tryptophan) Ionen.

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Occurence of Diploid Drones in a Neotropical Bumblebee

MACKENSEN[1] suggested that the sex determination mechanism of *Apis mellifera* is similar to that of *Habrobracon*. Diploid patches of male tissue were found[2] in *Apis mellifera* drones and diploid male larvae were detected[3] and subsequently reared to adult diploid males; details of cytology, biological importance, etc., are gradually being accumulated[4]. The possibility of obtaining bumblebee mating under controlled conditions has already been explored[5].

The present detection of diploid drones in *Bombus atratus* Franklin, the commonest bumblebee species in our area, is a byproduct of my main research on the reproductive biology of social bees.

Laboratory-mated bumblebee queens were placed individually into small observation cages containing pollen and honey and kept at 29°C to 30°C. One of them, mated on May 14, 1971, laid her first batch of eggs on the cage floor on June 23. In order to increase the possibility of successful nest foundation we added to the incipient colony several coccoons containing bumblebee worker pupae collected from our stock-colonies. In the meanwhile the queen oviposited 6 additional cells. At the proper time the introduced workers constructed the pockets but never provisioned them. All the necessary protein food was added in the form of honey-moistened pollen of *Apis mellifera* poured by us into the cell pockets. Of all eggs laid by this queen 7 workers and 10 males were obtained.

The first offspring to emerge (on July 31) was a male. When the male was 13 days old he mated with his mother and the same happened on August 20th with a second male, that emerged on August 11. The durations of the copulae were respectively 60 and 53 min. After this inbreeding the whole colony was transfered to a larger cage placed inside the laboratory. Through a hole in the wall the bees could fly and collect food freely. Henceforth the queen produced 14 workers and 27 males. Two light-pink eyed male pupae were used for chromosome counting (testicular smearing, aceto-orcein staining). SILVEIRA (personal commun.) counted n = 20 chromosomes for *B. atratus* (Figure 1); both drones here showed

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$n = 40$ at meiotic metaphase (Figure 2); it was concluded that they were both diploids. Another 8 adult diploid drones were used for sperm counting (Jaycox method)[6].

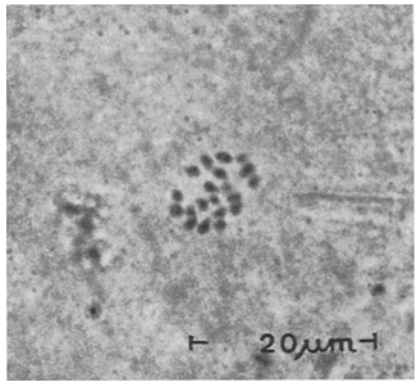

Fig. 1. Phase contrast photomicrography of spermatogonia of a normal (haploid) male of *Bombus atratus*, stained with aceto-orcein, showing 20 chromosomes (courtesy of Ms. Z. SILVEIRA).

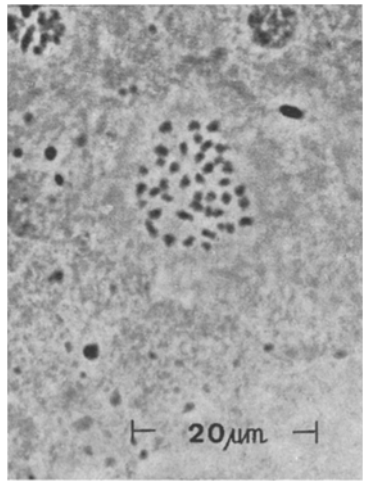

Fig. 2. Phase contrast photomicrography of spermatogonia showing 40 chromosomes of a diploid male son of a female *Bombus atratus* mated with her own son (same magnification as in Figure 1).

The number obtained, 300,000 to 770,000 sperms per drone (average = 522,500 ± 78,238,36) is non overlaping and distinctly smaller than the 1,301,000 to 3,030,000 (average = 1,982,000 ± 62,869,31) counted in a sample of 45 haploid *B. atratus* drones. Such deficient sperm production in diploids was also found by CHAUD (personal commun.) in *Apis mellifera* diploid drones.

If only 1 locus was involved, equal numbers of workers and diploid drones would be expected; the observed results differ significantly from this ($\varkappa_1^2 = 4.1$, $p < 0.05$). However, if 2 loci were involved, such that homozygotes at either in this experiment became males, then a 3 :1 ratio of diploid drones to workers would be expected and this is compatible with the data ($\varkappa_1^2 = 1.9$, n.s.), although other interpretations are possible.

If more of this rare event (cross of mother × son in *Bombus*) happen, more data will be obtained to thoroughly understand the case. Anyhow, the present work shows a further case of diploid drones in Hymenoptera, and indicates that this mechanism is older in Apidae than has been suggested[7].

Zusammenfassung. Mutter-Sohn-Kreuzung bei der südamerikanischen Hummel *Bombus atratus* Franklin ergab diploide Männchen mit normalen Arbeiterinnen im Verhältnis von 3 zu 1. Tur Interpretation des genetischen Mechanismus wurden 2 geschlechtsgebundene Loci angenommen, als bisher angenommen wurde, was für einen älteren Mechanismus bei Apiden spricht.

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Tissue Culture of *Cynodon dactylon*[1]

There is much information on culture of tissues from dicotyledons. However, similar information from monocotyledons is rather scarce; only a few species have been tissue cultured successfully: maize[2], ryegrass[3]; rye[4], sugarcane[5], oat[6,7], rice[8], lily[9], wheat[10], asparagus[11], sorghum[12]. This communication reports on the induction and growth of callus tissue from different parts of bermudagrass plant.

Apical sections of stolons including 3–4 nodes were surface sterilized in sodium hypochlorite solution and rinsed with sterilized water. Each section was divided into a) sheaths, b) nodes including 1–2 mm of the contiguous internodes and c) internodes ranging from 2 cm to full elongation. Inflorescences still enclosed in sheaths were also sterilized as described. Explants were implanted onto the nutrient medium.

The medium contained macroelements and vitamines according to WHITE[13], except that the concentration of $Na2HPO_4$ was 200 mg/l and Fe was supplied as FEDTA (35 mg/l); microelements were those used by TORREY[14]. The medium also contained sucrose (35 mg/l), NH_4NO_3 (500 mg/l) and myoinositol (50 mg/l). When supplemented with 2 mg/l 2,4-dichlorophenoxiacetic acid (2,4-D) this medium induced callus formation in sheaths, nodes, internodes and inflorescences. Naphthalene acetic acid (NAA) was less effective than 2,4-D as concentrations lower than 20 mg/l failed to induce callus formation.

Sheaths formed callus at the basal end. In inflorescences a detailed observation of the spikelets showed that callus was initiated at the basal part of the bracts. In internodes the ability to form callus appeared to depend on age (length) of explants. Young (short) internodes