$n = 40$ 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].

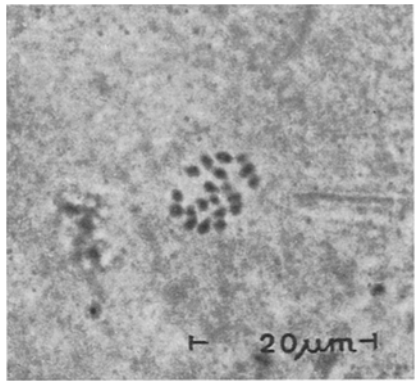


Fig. 1. Phase contrast photomicrography of spermatogonia of a normal (haploid) male of *Bombus atratus*, stained with aceto-orcein, showing 20 chromosomes (courtesy of Ms. Z. SILVEIRA).

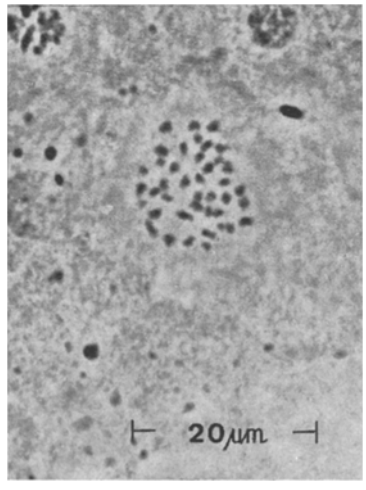


Fig. 2. Phase contrast photomicrography of spermatogonia showing 40 chromosomes of a diploid male son of a female *Bombus atratus* mated with her own son (same magnification as in Figure 1).

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.

C. A. GARÓFALO[8,9]

Departamento de Genética da Faculdade de Medicina de Ribeirão Prêto, Caixa Postal 301, 14100 Ribeirão Prêto São Paulo (Brazil), 6 November 1972.

[6] W. E. KERR, R. ZUCCHI, J. T. NAKADAIRA and J. E. BUTOLO, J. NY. ent. Soc. *70*, 265 (1962).
[7] W. E. KERR and R. A. NIELSEN, J. apic. Res. *6*, 3 (1967).
[8] This study was made possible by a grant of the State of São Paulo Research Foundation (FAPESP).
[9] Acknowledgment. I would like to thank Dr. W. E. KERR, Dr. R. ZUCCHI and Dr. S. F. SAKAGAMI for their excellent assistance during the course of this investigation as well during the translation. I also would like to thank Dr. R. H. CROZIER for his suggestions and comments and I extend my thankfulness to professors J. CHAUD NETTO and Z. SILVEIRA.

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

formed callus at both apical and basal ends, but, as internodes were more elongated, callus formation was restricted to the basal end (Figure). In fully elongated internodes, callus was rarely induced.

Callus from the different explants have been subcultured at monthly intervals through 46 transfers on to medium of similar composition to that which gave callus induction and subculturing is still in progress. A high callus growth rate was observed. Subcultured pieces initially weighed 200–250 mg and after a month their weight had increased approximately 10–15 fold. The cultures were maintained at 26 °C with 16 h of photoperiod; ilumination was 1500 lux fluorescent light.

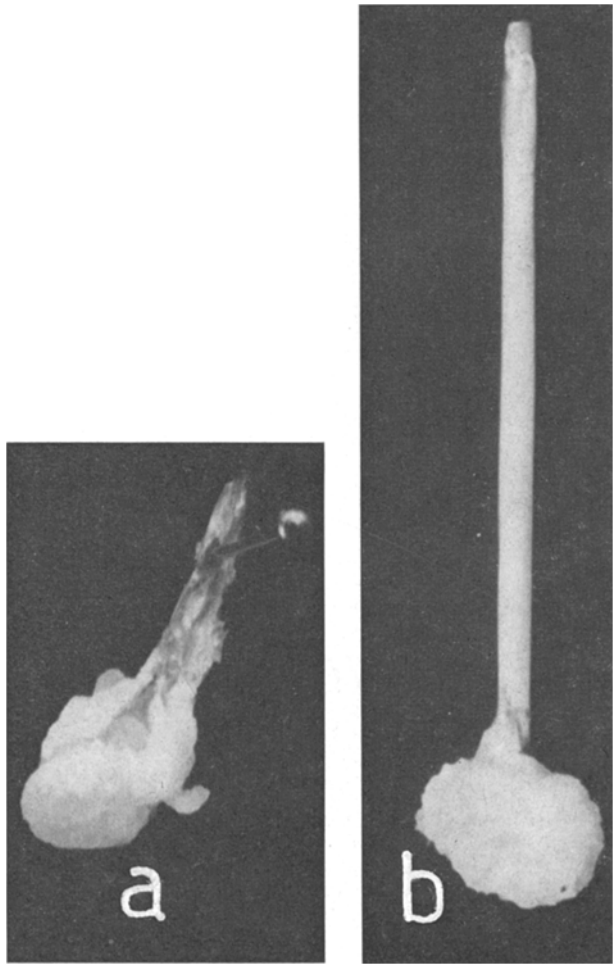
Callus from the different explants consisted of friable masses of tissue formed by cells of irregular shapes ranging from globular to tubular. No sign of organization was apparent in 1–2 week old callus growing in medium with 2 mg/l 2,4-D as revealed by microscopic examination, except that a few vessel cells had developed. Older callus (3–4 week old) frequently differentiated roots, most of which remained 3–4 mm long, but some continued growing. Roots contacting the medium formed, in turn, new callus. The number and length of roots increased in medium modified by lowering the 2,4-D level while callus growth rate decreased (Table). Without 2,4-D, roots developed but callus died in the following subcultures. Root differentiation was also observed when NAA was used instead of 2,4-D.

Effect of 2,4-D on callus growth and root differentiation

2,4-D	Growth	Root
2	+++	Ra
0.2	++	R
0.02	+	RR
0	—	R

+++, large callus; ++, medium callus; +, small callus. Number of R letters indicates amount of roots. Ra very short roots in some callus.

In attempts to induce shoot formation various concentrations of kinetin, 6-benzyl-adenine (BA), coconut water and cassein hydrolizate added to the medium (without 2,4-D) as well as combinations of these substances with different levels of 2,4-D and NAA. These trials were conducted with callus from the 4th, 28th and 39th subcultures. In no case did shoot differentiation occur. It was observed that BA at concentration between 0.3 mg/l and 1.2 mg/l induced a purple pigmentation in callus from the 4th subculture, both in the presence or absence of auxin (2,4-D or NAA). Kinetin at similar levels as that of BA failed to induce color development. The color appeared in a few restricted areas of the callus during the first days following transfers, but later the whole callus began to brown, with the process being more rapid in pigmented areas. No color was apparent in callus from the 28th and 39th subcultures, suggesting that tissue had lost the ability to produce pigmentation with time in culture. This resembles the decrease in the ability of callus to differentiate shoots as reported for callus tissues maintained in culture for long time periods (TORREY[15]; BARBA et al.[16]).



Callus formed at the basal end of a) sheaths b) internodes.

Resumen. Se indujo la formación de callos en nudos, entrenudos, vainas e inflorescencias de *Cynodon dactylon* (L.) Pers. en un nutritivo que contenía 2 mg/l de 2,4-D. Los callos se subcultivan desde hace tres años en el mismo medio. Cuando se disminuyó la concentración de 2,4-D se produjo diferenciación de raíces pero los intentos para diferenciar yemas no tuvieron éxito. La adición de 6-bencil-adenina al medico indujo una pigmentación roja en los tejidos.

H. L. MARODER and IMELDA A. PREGO

Unidad Fisiología Vegetal,
Centro de Investigaciones en Ciencias Agronómicas,
C.I.C.A., I.N.T.A., Castelar, Buenos Aires (Argentina),
5 December 1972.

[1] Publicación técnica Fis. No. 121, C.I.C.A.
[2] J. STRAUS and C. D. LARUE, Am. J. Bot. *41*, 687 (1954).
[3] K. J. NORSTOG, Bot. Gaz. *117*, 253 (1956).
[4] D. P. CAREW and A. E. SCHWARTING, Bot Gaz. *119*, 237 (1958).
[5] L. G. NICKELL, Hawaii. Plrs. Rec. *57*, 223 (1964).
[6] J. M. WEBSTER, Nature, Lond. *212*, 1472 (1966).
[7] O. CARTER, Y. YAMADA and E. TAKAHASHI, Nature, Lond. *214*, 1029 (1967).
[8] M. YATAZAWA, K. FURUHASHI and M. SHIMIZU, Pl. Cell Physiol., Tokyo *8*, 363 (1967).
[9] W. F. SHERIDAN, Planta *82*, 189 (1968).
[10] J. E. TRIONE, L. E. JONES and R. J. METZGER, Am. J. Bot. *55*, 529 (1968).
[11] C. WILMAR and L. M. HELLENDOORN, Nature, Lond. *217*, 369 (1968).
[12] V. J. MASTELLER and D. J. HOLDEN, Pl. Physiol. *45*, 362 (1970).
[13] P. R. WHITE, in *The cultivation of animal and plant cells*, 2nd edn. (Ronald Press, New York 1963).
[14] J. G. TORREY, Proc. natn. Acad. Sci., USA *43*, 887 (1957).
[15] J. G. TORREY, Physiologia. Pl. *20*, 265 (1967).
[16] R. BARBA and L. G. NICKELL, Planta *89*, 299 (1969).