

LAB SEVEN GENETICS OF ORGANISMS

OVERVIEW

In this lab you will use living organisms to do genetic crosses. You will learn how to collect and manipulate the organisms, collect data from F_1 and F_2 generations, and analyze the results from a monohybrid, dihybrid, or sex-linked cross. The procedures that follow apply to fruit flies; your teacher may substitute other procedures using other organisms.

OBJECTIVES

Before doing this lab you should understand:

- chi-square analysis of data, and
- the life cycle of diploid organisms useful in genetics studies.

After doing this lab you should be able to:

- investigate the independent assortment of two genes and determine whether the two genes are autosomal or sex-linked using a multigeneration experiment, and
- analyze the data from your genetic crosses using chi-square analysis techniques.

INTRODUCTION

Drosophila melanogaster, the fruit fly, is an excellent organism for genetics studies because it has simple food requirements, occupies little space, is hardy, completes its life cycle in about 12 days at room temperature, produces large numbers of offspring, can be immobilized readily for examination and sorting, and has many types of hereditary variations that can be observed with low-power magnification. *Drosophila* has a small number of chromosomes (four pairs). These chromosomes are easily located in the large salivary gland cells. *Drosophila* exists in stock cultures that can be readily obtained from several sources. Much research about the genetics of *Drosophila* during the last 50 years has resulted in a wealth of reference literature and a knowledge about hundreds of its genes.

The Life Cycle of *Drosophila*

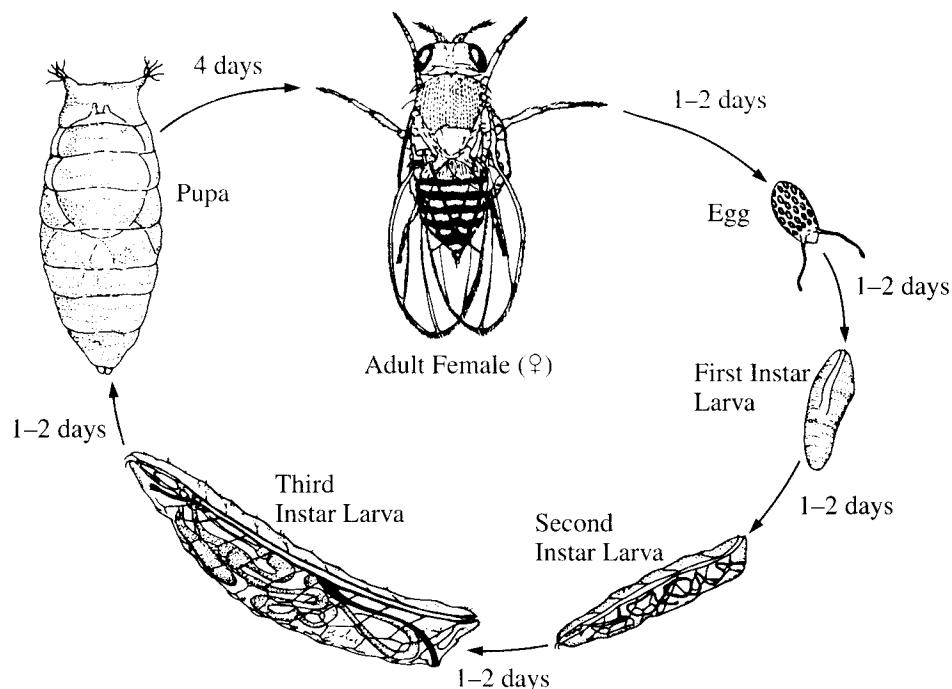
The Eggs. The eggs are small, oval shaped, and have two filaments at one end. They are usually laid on the surface of the culture medium and, with practice, can be seen with the naked eye. The eggs hatch into larvae after about a day.

The Larval Stage. The wormlike larva eats almost continuously, and its black mouth parts can easily be seen moving back and forth even when the larva itself is less distinct. Larvae tunnel through the culture medium while eating; thus, channels are a good indication of the successful growth of a culture. The larva sheds its skin twice as it increases in size. In the last of the three larval stages, the cells of the salivary glands contain giant chromosomes, which may be seen readily under low-power magnification after proper staining.

The Pupal Stage. When a mature larva in a lab culture is about to become a pupa, it usually climbs up the side of the culture bottle or on to the strip provided in the culture bottle. The last larval covering then becomes harder and darker, forming the pupal case. Through this case the later stages of metamorphosis to an adult fly can be observed. In particular, the eyes, the wings, and the legs become readily visible.

The Adult Stage. When metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of an adult fly. They live a month or more and then die. A female does not mate for about ten to twelve hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. To ensure a controlled mating, it is necessary to use females that have not mated before (virgins).

Figure 7.1: The Life Cycle of *Drosophila melanogaster*



It is important to realize that a number of factors determine the length of time of each stage in the life cycle. Of these factors, temperature is the most important. At room temperature (about 25°C), the complete cycle takes ten to twelve days.

Design of the Exercise

This genetics experiment will be carried on for several weeks. *Drosophila* with well-defined mutant traits will be assigned to you by your teacher. You are responsible for making observations and keeping records concerning what happens as mutant traits are passed from one generation to the next.

You will be assigned to study a certain mode of inheritance using particular genetic crosses of flies having one or two mutations. The modes of inheritance most commonly used are:

1. **Monohybrid.** In these experiments the mode of inheritance is determined when a single contrasting pair of characteristics is involved.
2. **Dihybrid.** In these experiments the mode of inheritance is determined when two pairs of contrasting characteristics are considered simultaneously.
3. **Sex-linked.** In these experiments the mode of inheritance is determined when the mutant characteristic is associated with the X-chromosome.

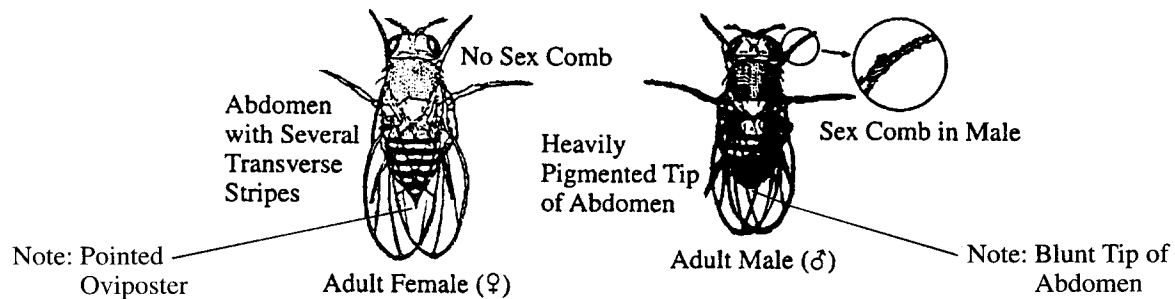
To make these experiments interesting and challenging, you will not be told the mode of inheritance, nor the name for the particular mutation(s) you are studying. Study the wild type flies (both male and female) until their phenotypic characteristics are familiar. Flies having one or two mutations can then be identified by making comparisons with the wild type flies. The most commonly studied mutations are in eye color or shape, bristle number or shape, wing size or shape, or antenna size or shape. You should make up your own name for the particular mutation(s) that you identify in your flies.

Procedure

1. Obtain a vial of wild type flies. Practice immobilizing and sexing (determining the gender of) these flies. Examine these flies and note the characteristics of their eyes, wings, bristles, and antennae.
2. To make handling easier, immobilize the flies by chilling them. Since the activity level of the flies is dependent on environmental temperature, the following steps immobilize the flies. (Your teacher may assign a different method.)
 - a. Hold the vial containing the flies at an angle and twirl it in ice for several minutes.
 - b. When the flies are immobilized, dump them into a small, plastic petri dish containing a #1 Whatman filter paper.
 - c. Place the petri dish on top of the ice in order to maintain the cool temperature necessary to keep the flies immobilized.
 - d. Use the dissecting microscope to view the flies. The top of the petri dish can be on or off when viewing.
3. Distinguish male flies from female flies by looking for the following characteristics (illustrated in Figure 7.2):
 - a. Males are usually smaller than females.
 - b. Males have dark, blunt abdomens, and females have lighter, pointed abdomens.
 - c. Only the males have sex combs, which are groups of black bristles on the uppermost joint of the forelegs.

♂ male - 1st 2nd 3rd 4th 5th 6th 7th 8th 9th 10th 11th 12th 13th 14th 15th 16th 17th 18th 19th 20th 21st 22nd 23rd 24th 25th 26th 27th 28th 29th 30th 31st 32nd 33rd 34th 35th 36th 37th 38th 39th 40th 41st 42nd 43rd 44th 45th 46th 47th 48th 49th 50th 51st 52nd 53rd 54th 55th 56th 57th 58th 59th 60th 61st 62nd 63rd 64th 65th 66th 67th 68th 69th 70th 71st 72nd 73rd 74th 75th 76th 77th 78th 79th 80th 81st 82nd 83rd 84th 85th 86th 87th 88th 89th 90th 91st 92nd 93rd 94th 95th 96th 97th 98th 99th 100th 101st 102nd 103rd 104th 105th 106th 107th 108th 109th 110th 111th 112th 113th 114th 115th 116th 117th 118th 119th 120th 121st 122nd 123rd 124th 125th 126th 127th 128th 129th 130th 131st 132nd 133rd 134th 135th 136th 137th 138th 139th 140th 141st 142nd 143rd 144th 145th 146th 147th 148th 149th 150th 151st 152nd 153rd 154th 155th 156th 157th 158th 159th 160th 161st 162nd 163rd 164th 165th 166th 167th 168th 169th 170th 171st 172nd 173rd 174th 175th 176th 177th 178th 179th 180th 181st 182nd 183rd 184th 185th 186th 187th 188th 189th 190th 191st 192nd 193rd 194th 195th 196th 197th 198th 199th 200th 201st 202nd 203rd 204th 205th 206th 207th 208th 209th 210th 211st 212th 213th 214th 215th 216th 217th 218th 219th 220th 221st 222nd 223rd 224th 225th 226th 227th 228th 229th 230th 231st 232nd 233rd 234th 235th 236th 237th 238th 239th 240th 241st 242nd 243rd 244th 245th 246th 247th 248th 249th 250th 251st 252nd 253rd 254th 255th 256th 257th 258th 259th 260th 261st 262nd 263rd 264th 265th 266th 267th 268th 269th 270th 271st 272nd 273rd 274th 275th 276th 277th 278th 279th 280th 281st 282nd 283rd 284th 285th 286th 287th 288th 289th 290th 291st 292nd 293rd 294th 295th 296th 297th 298th 299th 300th 301st 302nd 303rd 304th 305th 306th 307th 308th 309th 310th 311st 312th 313th 314th 315th 316th 317th 318th 319th 320th 321st 322nd 323rd 324th 325th 326th 327th 328th 329th 330th 331st 332nd 333rd 334th 335th 336th 337th 338th 339th 340th 341st 342nd 343rd 344th 345th 346th 347th 348th 349th 350th 351st 352nd 353rd 354th 355th 356th 357th 358th 359th 360th 361st 362nd 363rd 364th 365th 366th 367th 368th 369th 370th 371st 372nd 373rd 374th 375th 376th 377th 378th 379th 380th 381st 382nd 383rd 384th 385th 386th 387th 388th 389th 390th 391st 392nd 393rd 394th 395th 396th 397th 398th 399th 400th 401st 402nd 403rd 404th 405th 406th 407th 408th 409th 410th 411st 412th 413th 414th 415th 416th 417th 418th 419th 420th 421st 422nd 423rd 424th 425th 426th 427th 428th 429th 430th 431st 432nd 433rd 434th 435th 436th 437th 438th 439th 440th 441st 442nd 443rd 444th 445th 446th 447th 448th 449th 450th 451st 452nd 453rd 454th 455th 456th 457th 458th 459th 460th 461st 462nd 463rd 464th 465th 466th 467th 468th 469th 470th 471st 472nd 473rd 474th 475th 476th 477th 478th 479th 480th 481st 482nd 483rd 484th 485th 486th 487th 488th 489th 490th 491st 492nd 493rd 494th 495th 496th 497th 498th 499th 500th 501st 502nd 503rd 504th 505th 506th 507th 508th 509th 510th 511st 512th 513th 514th 515th 516th 517th 518th 519th 520th 521st 522nd 523rd 524th 525th 526th 527th 528th 529th 530th 531st 532nd 533rd 534th 535th 536th 537th 538th 539th 540th 541st 542nd 543rd 544th 545th 546th 547th 548th 549th 550th 551st 552nd 553rd 554th 555th 556th 557th 558th 559th 560th 561st 562nd 563rd 564th 565th 566th 567th 568th 569th 570th 571st 572nd 573rd 574th 575th 576th 577th 578th 579th 580th 581st 582nd 583rd 584th 585th 586th 587th 588th 589th 590th 591st 592nd 593rd 594th 595th 596th 597th 598th 599th 600th 601st 602nd 603rd 604th 605th 606th 607th 608th 609th 610th 611st 612th 613th 614th 615th 616th 617th 618th 619th 620th 621st 622nd 623rd 624th 625th 626th 627th 628th 629th 630th 631st 632nd 633rd 634th 635th 636th 637th 638th 639th 640th 641st 642nd 643rd 644th 645th 646th 647th 648th 649th 650th 651st 652nd 653rd 654th 655th 656th 657th 658th 659th 660th 661st 662nd 663rd 664th 665th 666th 667th 668th 669th 670th 671st 672nd 673rd 674th 675th 676th 677th 678th 679th 680th 681st 682nd 683rd 684th 685th 686th 687th 688th 689th 690th 691st 692nd 693rd 694th 695th 696th 697th 698th 699th 700th 701st 702nd 703rd 704th 705th 706th 707th 708th 709th 710th 711st 712th 713th 714th 715th 716th 717th 718th 719th 720th 721st 722nd 723rd 724th 725th 726th 727th 728th 729th 730th 731st 732nd 733rd 734th 735th 736th 737th 738th 739th 740th 741st 742nd 743rd 744th 745th 746th 747th 748th 749th 750th 751st 752nd 753rd 754th 755th 756th 757th 758th 759th 760th 761st 762nd 763rd 764th 765th 766th 767th 768th 769th 770th 771st 772nd 773rd 774th 775th 776th 777th 778th 779th 780th 781st 782nd 783rd 784th 785th 786th 787th 788th 789th 790th 791st 792nd 793rd 794th 795th 796th 797th 798th 799th 800th 801st 802nd 803rd 804th 805th 806th 807th 808th 809th 810th 811st 812th 813th 814th 815th 816th 817th 818th 819th 820th 821st 822nd 823rd 824th 825th 826th 827th 828th 829th 830th 831st 832nd 833rd 834th 835th 836th 837th 838th 839th 840th 841st 842nd 843rd 844th 845th 846th 847th 848th 849th 850th 851st 852nd 853rd 854th 855th 856th 857th 858th 859th 860th 861st 862nd 863rd 864th 865th 866th 867th 868th 869th 870th 871st 872nd 873rd 874th 875th 876th 877th 878th 879th 880th 881st 882nd 883rd 884th 885th 886th 887th 888th 889th 890th 891st 892nd 893rd 894th 895th 896th 897th 898th 899th 900th 901st 902nd 903rd 904th 905th 906th 907th 908th 909th 910th 911st 912th 913th 914th 915th 916th 917th 918th 919th 920th 921st 922nd 923rd 924th 925th 926th 927th 928th 929th 930th 931st 932nd 933rd 934th 935th 936th 937th 938th 939th 940th 941st 942nd 943rd 944th 945th 946th 947th 948th 949th 950th 951st 952nd 953rd 954th 955th 956th 957th 958th 959th 960th 961st 962nd 963rd 964th 965th 966th 967th 968th 969th 970th 971st 972nd 973rd 974th 975th 976th 977th 978th 979th 980th 981st 982nd 983rd 984th 985th 986th 987th 988th 989th 990th 991st 992nd 993rd 994th 995th 996th 997th 998th 999th 1000th 1001st 1002nd 1003rd 1004th 1005th 1006th 1007th 1008th 1009th 1010th 1011st 1012th 1013th 1014th 1015th 1016th 1017th 1018th 1019th 1020th 1021st 1022nd 1023rd 1024th 1025th 1026th 1027th 1028th 1029th 1030th 1031st 1032nd 1033rd 1034th 1035th 1036th 1037th 1038th 1039th 1040th 1041st 1042nd 1043rd 1044th 1045th 1046th 1047th 1048th 1049th 1050th 1051st 1052nd 1053rd 1054th 1055th 1056th 1057th 1058th 1059th 1060th 1061st 1062nd 1063rd 1064th 1065th 1066th 1067th 1068th 1069th 1070th 1071st 1072nd 1073rd 1074th 1075th 1076th 1077th 1078th 1079th 1080th 1081st 1082nd 1083rd 1084th 1085th 1086th 1087th 1088th 1089th 1090th 1091st 1092nd 1093rd 1094th 1095th 1096th 1097th 1098th 1099th 1100th 1101st 1102nd 1103rd 1104th 1105th 1106th 1107th 1108th 1109th 1110th 1111st 1112th 1113th 1114th 1115th 1116th 1117th 1118th 1119th 1120th 1121st 1122nd 1123rd 1124th 1125th 1126th 1127th 1128th 1129th 1130th 1131st 1132nd 1133rd 1134th 1135th 1136th 1137th 1138th 1139th 1140th 1141st 1142nd 1143rd 1144th 1145th 1146th 1147th 1148th 1149th 1150th 1151st 1152nd 1153rd 1154th 1155th 1156th 1157th 1158th 1159th 1160th 1161st 1162nd 1163rd 1164th 1165th 1166th 1167th 1168th 1169th 1170th 1171st 1172nd 1173rd 1174th 1175th 1176th 1177th 1178th 1179th 1180th 1181st 1182nd 1183rd 1184th 1185th 1186th 1187th 1188th 1189th 1190th 1191st 1192nd 1193rd 1194th 1195th 1196th 1197th 1198th 1199th 1200th 1201st 1202nd 1203rd 1204th 1205th 1206th 1207th 1208th 1209th 1210th 1211st 1212th 1213th 1214th 1215th 1216th 1217th 1218th 1219th 1220th 1221st 1222nd 1223rd 1224th 1225th 1226th 1227th 1228th 1229th 1230th 1231st 1232nd 1233rd 1234th 1235th 1236th 1237th 1238th 1239th 1240th 1241st 1242nd 1243rd 1244th 1245th 1246th 1247th 1248th 1249th 1250th 1251st 1252nd 1253rd 1254th 1255th 1256th 1257th 1258th 1259th 1260th 1261st 1262nd 1263rd 1264th 1265th 1266th 1267th 1268th 1269th 1270th 1271st 1272nd 1273rd 1274th 1275th 1276th 1277th 1278th 1279th 1280th 1281st 1282nd 1283rd 1284th 1285th 1286th 1287th 1288th 1289th 1290th 1291st 1292nd 1293rd 1294th 1295th 1296th 1297th 1298th 1299th 1300th 1301st 1302nd 1303rd 1304th 1305th 1306th 1307th 1308th 1309th 1310th 1311st 1312th 1313th 1314th 1315th 1316th 1317th 1318th 1319th 1320th 1321st 1322nd 1323rd 1324th 1325th 1326th 1327th 1328th 1329th 1330th 1331st 1332nd 1333rd 1334th 1335th 1336th 1337th 1338th 1339th 1340th 1341st 1342nd 1343rd 1344th 1345th 1346th 1347th 1348th 1349th 1350th 1351st 1352nd 1353rd 1354th 1355th 1356th 1357th 1358th 1359th 1360th 1361st 1362nd 1363rd 1364th 1365th 1366th 1367th 1368th 1369th 1370th 1371st 1372nd 1373rd 1374th 1375th 1376th 1377th 1378th 1379th 1380th 1381st 1382nd 1383rd 1384th 1385th 1386th 1387th 1388th 1389th 1390th 1391st 1392nd 1393rd 1394th 1395th 1396th 1397th 1398th 1399th 1400th 1401st 1402nd 1403rd 1404th 1405th 1406th 1407th 1408th 1409th 1410th 1411st 1412th 1413th 1414th 1415th 1416th 1417th 1418th 1419th 1420th 1421st 1422nd 1423rd 1424th 1425th 1426th 1427th 1428th 1429th 1430th 1431st 1432nd 1433rd 1434th 1435th 1436th 1437th 1438th 1439th 1440th 1441st 1442nd 1443rd 1444th 1445th 1446th 1447th 1448th 1449th 1450th 1451st 1452nd 1453rd 1454th 1455th 1456th 1457th 1458th 1459th 1460th 1461st 1462nd 1463rd 1464th 1465th 1466th 1467th 1468th 1469th 1470th 1471st 1472nd 1473rd 1474th 1475th 1476th 1477th 1478th 1479th 1480th 1481st 1482nd 1483rd 1484th 1485th 1486th 1487th 1488th 1489th 1490th 1491st 1492nd 1493rd 1494th 1495th 1496th 1497th 1498th 1499th 1500th 1501st 1502nd 1503rd 1504th 1505th 1506th 1507th 1508th 1509th 1510th 1511st 1512th 1513th 1514th 1515th 1516th 1517th 1518th 1519th 1520th 1521st 1522nd 1523rd 1524th 1525th 1526th 1527th 1528th 1529th 1530th 1531st 1532nd 1533rd 1534th 1535th 1536th 1537th 1538th 1539th 1540th 1541st 1542nd 1543rd 1544th 1545th 1546th 1547th 1548th 1549th 1550th 1551st 1552nd 1553rd 1554th 1555th 1556th 1557th 1558th 1559th 1560th 1561st 1562nd 1563rd 1564th 1565th 1566th 1567th 1568th 1569th 1570th 1571st 1572nd 1573rd 1574th 1575th 1576th 1577th 1578th 1579th 1580th 1581st 1582nd 1583rd 1584th 1585th 1586th 1587th 1588th 1589th 1590th 1591st 1592nd 1593rd 1594th 1595th 1596th 1597th 1598th 1599th 1600th 1601st 1602nd 1603rd 1604th 1605th 1606th 1607th 1608th 1609th 1610th 1611st 1612th 1613th 1614th 1615th 1616th 1617th 1618th 1619th 1620th 1621st 1622nd 1623rd 1624th 1625th 1626th 1627th 1628th 1629th 1630th 1631st 1632nd 1633rd 1634th 1635th 1636th 1637th 1638th 1639th 1640th 1641st 1642nd 1643rd 1644th 1645th 1646th 1647th 1648th 1649th 1650th 1651st 1652nd 1653rd 1654th 1655th 1656th 1657th 1658th 1659th 1660th 1661st 1662nd 1663rd 1664th 1665th 1666th 1667th 1668th 1669th 1670th 1671st 1672nd 1673rd 1674th 1675th 1676th 1677th 1678th 1679th 1680th 1681st 1682nd 1683rd 1684th 1685th 1686th 1687th 1688th 1689th 1690th 1691st 1692nd 1693rd 1694th 1695th 1696th 1697th 1698th 1699th 1700th 1701st 1702nd 1703rd 1704th 1705th 1706th 1707th 1708th 1709th 1710th 1711st 1712th 1713th 1714th 1715th 1716th 1717th 1718th 1719th 1720th 1721st 1722nd 1723rd 1724th 1725th 1726th 1727th 1728th 1729th 1730th 1731st 1732nd 1733rd 1734th 1735th 1736th 1737th 1738th 1739th 1740th 1741st 1742nd 1743rd 1744th 1745th 1746th 1747th 1748th 1749th 1750th 1751st 1752nd 1753rd 1754th 1755th 1756th 1757th 1758th 1759th 1760th 1761st 1762nd 1763rd 1764th 1765th 1766th 1767th 1768th 1769th 1770th 1771st 1772nd 1773rd 1774th 1775th 1776th 1777th 1778th 1779th 1780th 1781st 1782nd 1783rd 1784th 1785th 1786th 1787th 1788th 1789th 1790th 1791st 1792nd 1793rd 1794th 1795th 1796th 1797th 1798th 1799th 1800th 1801st 1802nd 1803rd 1804th 1805th 1806th 1807th 1808th 1809th 1810th 1811st 1812th 1813th 1814th 1815th 1816th 1817th 1818th 1819th 1820th 1821st 1822nd 1823rd 1824th 1825th 1826th 1827th 1828th 1829th 1830th 1831st 1832nd 1833rd 1834th 1835th 1836th 1837th 1838th 1839th 1840th 1841st 1842nd 1843rd 1844th 1845th 1846th 1847th 1848th 1849th 1850th 1851st 1852nd 1853rd 1854th 1855th 1856th 1857th 1858th 1859th 1860th 1861st 1862nd 1863rd 1864th 1865th 1866th 1867th 1868th 1869th 1870th 1871st 1872nd 1873rd 1874th 1875th 1876th 1877th 1878th 1879th 1880th 1881st 1882nd 1883rd 1884th 1885th 1886th 1887th 1888th 1889th 1890th 1891st 1892nd 1893rd 1894th 1895th 1896th 1897th 1898th 1899th 1900th 1901st 1902nd 1903rd 1904th 1905th 1906th 1907th 1908th 1909th 1910th 1911st 1912th 1913th 1914th 1915th 1916th 1917th 1918th 1919th 1920th 1921st 1922nd 1923rd 1924th 1925th 1926th 1927th 1928th 1929th 1930th 1931st 1932nd 1933rd 1934th 1935th 1936th 1937th 1938th 1939th 1940th 1941st 1942nd 1943rd 1944th 1945th 1946th 1947th 1948th 1949th 1950th 1951st 1952nd 1953rd 1954th 1955th 1956th 1957th 1958th 1959th 1960th 1961st 1962nd 1963rd 1964th 1965th 1966th 1967th 1968th 1969th 1970th 1971st 1972nd 1973rd 1974th 1975th 1976th 1977th 1978th 1979th 1980th 1981st 1982nd 1983rd 1984th 1985th 1986th 1987th 1988th 1989th 1990th 1991st 1992nd 1993rd 1994th 1995th 1996th 1997th 1998th 1999th 2000th 2001st 2002nd 2003rd 2004th 2005th 2006th 2007th 2008th 2009th 2010th 2011st 2012th 2013th 2014th 2015th 2016th 2017th 2018th 2019th 2020th 2021st 2022nd 2023rd 2024th 2025th 2026th 2027th 2028th 2029th 2030th 2031st 2032nd 2033rd 2034th 2035th 2036th 2037th 2038th 2039th 2040th 2041st 2042nd 2043rd 2044th 2045th 2046th 2047th 2048th 2049th 2050th 2051st 2052nd 2053rd 2054th 2055th 2056th 2057th 2058th 2059th 2060th 2061st 2062nd 2063rd 2064th 2065th 2066th 2067th 2068th 2069th 2070th 2071st 2072nd 2073rd 2074th 2075th 2076th 2077th 2078th 2079th 2080th 2081st 2082nd 2083rd 2084th 2085th 2086th 2087th 2088th 2089th 2090th 2091st 2092nd 2093rd 2094th 2095th 2096th 2097th 2098th 2099th 2100th 2101st 2102nd 2103rd 2104th 2105th 2106th 2107th 2108th 2109th 2110th 2111st 2112th 2113th 2114th 2

Figure 7.2: Female and Male *Drosophila*



4. Obtain a vial containing pairs of experimental flies. Record the cross number of the vial. This number will serve as a record as to which cross you have obtained. These flies are the parental generation (P) and have already mated. The females should have already laid eggs on the surface of the culture medium. The eggs (or maybe larvae now) represent the first filial, F_1 , generation and will be emerging from their pupal cases in about a week.
5. **First Week (Today).** Immobilize and remove the adult flies. Observe them carefully under the dissecting microscope. Separate the males from the females and look for the mutation(s). Note whether the mutation(s) is/are associated with the males or the females. Identify the mutation(s) and give it/them a made-up name and symbol. Record the phenotype and symbol in Table 7.1. The findings should be confirmed by your teacher.
6. Place the parents in the morgue (a jar containing alcohol or baby oil). Label the vial containing the eggs or larvae with symbols for the mating. For example, if a sepia-eyed female is crossed with a wild-type male, the label could be "sepia ♀ X wild ♂". Also be sure to label the vial with your name and the date. Place the vial in a warm location.
7. **Second Week.** Begin by observing the F_1 flies. Immobilize and examine all the flies. Record their sex and the presence or absence of the mutation(s) (as observed in the parental flies) in Table 7.1. Consider the conclusions that can be drawn from these data. Place 5 or 6 pairs of F_1 flies in a fresh culture bottle and the rest of the flies in the morgue. For this cross the females need not be virgins. Label the new vial " F_1 X F_1 ". Also, label the vial with symbols denoting the cross, the date, and your name.
8. **Third Week.** Remove the F_1 flies from the vials and place them in the morgue. The F_2 generation are the eggs and/or larvae in the vial. Place the vial back in the warm location.
9. **Fourth Week.** Begin removing the F_2 flies. Record their sex and the presence or absence of the mutant phenotypes (as observed in the parental flies in Table 7.2). The more F_2 flies collected, the more reliable the data will be. You may have to collect flies over a 3- or 4-day period. Try to collect at least 200 flies.
10. To analyze your data, you will need to learn how to use the chi-square test. Go to the Statistical Analysis Section (page 85) to review this technique.



Table 7.1: F₁ Generation Data

Date _____

Phenotype and Symbol	Females	Males

Table 7.2: F₂ Generation Data

Date _____

Phenotype and Symbol	Females	Males

Analysis of Results

1. Describe and name the observed mutation(s).



6. From the results, describe your cross.

Is the mutation sex-linked or autosomal? _____

Is the mutation a dominant or recessive? _____

Is the cross monohybrid or dihybrid? _____

7. Are the deviations for the phenotypic ratio of the F_2 generation within the limits expected by chance? To answer this question, statistically analyze the data using the chi-square analysis. Calculate the chi-square statistic for the F_2 generation in the chart below. Refer to the critical values of the chi-square (χ^2) distribution table (Table 7.5 on page 86) to determine the p (probability) value that is associated with your χ^2 statistic.

Phenotype	# Observed (o)	# Expected (e)	(o-e)	(o-e) ²	$\frac{(o-e)^2}{e}$
$\chi^2 =$					

a. Calculate the chi-square value for these data.

1. How many degrees of freedom are there? _____

2. chi-square (χ^2) = _____

3. Referring to the critical values chart, what is the probability value for this data?

b. According to the probability value, can you accept or reject your null hypothesis?
Explain why.



Topics for Discussion

1. Why was it necessary for the females of the parental generation to be virgins?

2. Why was it not necessary to isolate virgin females for the F_1 cross?

3. Why were the adult flies removed from the vials at weeks 2 and 4?

LAB SEVEN STATISTICAL ANALYSIS SECTION

Using the Chi-Square Test for Statistical Analysis of Experimental Data

Example 1

Statistics can be used to determine if differences among groups are significant, or simply the result of predictable error. The statistical test most frequently used to determine whether data obtained experimentally provide a good fit or approximation to the expected or theoretical data is the chi-square test. This test can be used to determine if deviations from the expected values are due to chance alone, or to some other circumstance. For example, consider corn seedlings resulting from an F_1 cross between parents that are heterozygous for color.

A Punnett square of the F_1 cross **Gg X Gg** would predict that the expected proportion of green:albino seedlings would be 3:1. Use this information to fill in the Expected (e) column and the (o-e) column in Table 7.3.

Table 7.3

Phenotype	Genotype	# Observed (o)	# Expected (e)	(o - e)
Green	GG or Gg	72		
Albino	gg	12		
Total:		84		

There is a small difference between the observed and expected results, but are these data close enough that the difference can be explained by random chance or variation in the sample?

To determine if the observed data fall within acceptable limits, a chi-square analysis is performed to test the validity of a **null hypothesis** (that there is no statistically significant difference between the observed and expected data). If the chi-square analysis indicates that the data vary too much from the expected 3:1, an **alternative hypothesis** is accepted.

The formula for chi-square is:

$$\chi^2 = \sum \frac{(o - e)^2}{e}$$

where **o** = **observed** number of individuals

e = **expected** number of individuals

Σ = the **sum of the values** (in this case, the differences, squared, divided by the number expected)

1. This statistical test will examine the null hypothesis, which predicts that the data from the experimental cross above will be expected to fit the 3:1 ratio.
2. Use the data from Table 7.3 to complete Table 7.4.

Table 7.4

Phenotype	# Observed (o)	# Expected (e)	(o - e)	(o - e) ²	$\frac{(o - e)^2}{e}$
Green	72				
Albino	12				
$\chi^2 = \sum \frac{(o - e)^2}{e} =$					

3. Your calculations should give you a value for $\chi^2 = 5.14$. This value is then compared to Table 7.5.

Table 7.5: Critical Values of the Chi-Square Distribution

Probability (p)	Degrees of Freedom (df)				
	1	2	3	4	5
0.05	3.84	5.99	7.82	9.49	11.1
0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

How to Use the Critical Values Table

1. Determine the **degrees of freedom (df)** for your experiment. It is the number of phenotypic classes minus 1. Since there are two possible genotypes, for this experiment $df = 1$ (2 samples -1). If the experiment had gathered data for a dihybrid cross, there would be four possible phenotypes and therefore 3 degrees of freedom.
2. Find the p value. Under the 1 df column, find the critical value in the probability (p) = 0.05 row: it is 3.84. What does this mean? **If the calculated chi-square value is greater than or equal to the critical value from the table, then the null hypothesis is rejected.** Since for our example $\chi^2 = 5.14$ and $5.14 > 3.84$, we reject our null hypothesis that there is no statistically significant difference between the observed and expected data. In other words, chance alone cannot explain the deviations we observed and there is, therefore, reason to doubt our original hypothesis (or to question our data collection accuracy.) The minimum probability for rejecting a null hypothesis in the sciences is generally 0.05, so this is the row to use in the chi-square table.
3. These results are said to be significant at a probability of $p = 0.05$. This means that only 5% of the time would you expect to see similar data if the null hypothesis was correct; thus, you are 95% sure that the data do not fit a 3:1 ratio.
4. Since these data do not fit the expected 3:1 ratio, you must consider reasons for this variation. Additional experimentation would be necessary. Perhaps the sample size is too small, or errors were made in data collection. In this example, perhaps the albino seedlings are underrepresented because they died before the counting was performed.

Example 2

In a study of incomplete dominance in tobacco seedlings, the counts in Table 7.6 were made from a cross between two heterozygous (Gg) plants:

Table 7.6

Phenotype	Genotype	# Observed (O)
Green	GG	22
Yellow Green	Gg	50
Albino	gg	12
Total:		84

A Punnett square for this cross indicates that the expected counts should be in a 1 green:2 yellow green:1 albino ratio (Table 7.7). The expected values for a total count of 84 organisms are therefore:

$$1 \text{ green} = \frac{1}{4} \times 84 = 21$$

$$2 \text{ yellow green} = \frac{1}{2} \times 84 = 42$$

$$1 \text{ yellow} = \frac{1}{4} \times 84 = \frac{21}{84}$$

Table 7.7

Phenotype	# Observed (o)	# Expected (e)	(o - e)	(o - e) ²	$\frac{(o - e)^2}{e}$
Green	22	21	1	1	0.05
Yellow Green	50	42	8	64	1.52
Albino	12	21	9	81	3.86
$\chi^2 = \sum \frac{(o - e)^2}{e} =$					5.43

Go to the chi-square table, this time for two degrees of freedom (there are three phenotypes: $3 - 1 = 2$ df). If the χ^2 value were greater than or equal to the critical value of 5.99 we would reject our hypothesis. Since 5.43 is less than the critical value at $p = .05$, we accept the null hypothesis (this second data set does fit the expected 1:2:1 ratio).

Practice Problem

An investigator observes that when pure-breeding, long-wing *Drosophila* are mated with pure-breeding, short-wing flies, the F_1 offspring have an intermediate wing length.

When several intermediate-wing-length flies are allowed to interbreed the following results are obtained:

Observed

230 long wings
510 intermediate-length wings
260 short wings

- What is the genotype of the F_1 intermediate-wing-length flies? _____
- Write a hypothesis describing the mode of inheritance of wing length in *Drosophila* (this is your null hypothesis).

c. Complete Table 7.8.

Table 7.8

Phenotype	# Observed (o)	# Expected (e)	(o - e)	(o - e) ²	$\frac{(o - e)^2}{e}$
$\chi^2 = \sum \frac{(o - e)^2}{e} =$					

(i) Calculate the chi-square value for these data.

1. How many degrees of freedom (df) are there? _____
2. χ^2 (chi-square) = _____
3. Referring to the critical values chart, what is the probability value for these data?

(ii) According to the critical value of χ^2 , can you accept or reject the null hypothesis?
Explain why.
