Microscopic Procedures for Plant Meiosis

James P. Braselton

The need for an understanding of meiosis by both beginning and experienced biologists is exemplified by the number of recent articles about techniques for improving the teaching of meiosis (Kindfield 1994; Levy & Benner 1995). Although the excellent diagrams that appear in most textbooks adequately combine information about DNA structure, pairing of homologous chromosomes, crossing-over between non-sister chromatids, and light microscopic views of chromosomal organization, many students and colleagues with whom I have discussed meiosis over the past 27 years often miss major aspects of meiosis because they are not familiar with how microscopic preparations of meiosis are made.

A major advantage of using freshly prepared slides of meiosis is that students critically examine the slides, and are able to compare meiotic to mitotic chromosomes in addition to observing stages of meiosis. It is not unusual to have students express amazement at how different the bivalents in metaphase I actually look when compared to diagrams from textbooks, and the structural differences between mitotic and meiotic chromosomes.

The value of using fresh or fixed flowers for observing meiosis in introductory biology classrooms was emphasized by articles about *Chlorophytum comosum* (Thunb.) Jacq. f "spider plant" (Chen & Gregory 1973), *Rhoeo discolor* (L'Her.) Hance (syn. = *Tradescantia spathacea* Sw.) "Moses-in-the-basket" (Satterfield & Mertens 1972), *Vicia faba* L. "broad bean" (Bempong 1973), and *Allium shoenoprasum* L. "chives" (Robinson 1985/86). The present communication expands on these articles, and is intended to serve as a guide for preparing slides of meiosis from flowering plants for individuals who teach meiosis in introductory biology or introductory cell biology at the college level.

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The techniques described here are derived from a variety of sources, but mainly have been modified in my laboratory from methods I learned while a graduate student under the direction of Professor C.C. Bowen at Iowa State University in the late 1960s, and while on sabbatical in 1978–79 in Professor Michael D. Bennett's laboratory when he was at the Plant Breeding Institute, Cambridge, UK, prior to his appointment as Director of Jodrell Laboratories at Kew Gardens. The methods are used routinely in our departmental introductory cell biology course. For individuals with limited experience in plant cytogenetics, I recommend Singh (1993) as a starting point for detailed descriptions of plant cytogenetic methods, and Rhoades (1961) and Moens (1987) for reviews of meiosis.

Selection of Plants

In flowering plants, megaspores are produced by meiosis within ovules in the ovaries, and develop into the embryo sac (megagametophyte). Because only one megasporocyte per ovule goes through meiosis, using ovules to illustrate meiosis is impractical. Microspores are produced by meiosis in anthers, and develop into microgametophytes (pollen). Anthers are an excellent source of meiotic material because they are easily accessible to students, and many cells in each anther go through meiosis. In some plants, meiotic divisions in all the anthers within a flower are relatively synchronous, whereas in others there is a range of development within one flower.

An important point for consideration when selecting a flowering plant for meiotic studies is the ease with which flowers that contain meiotic stages may be located. Because the novice generally selects buds with mature pollen, it is desirable to use plants with inflorescences with a gradation of maturity of flowers; the investigator then may work from immature toward mature buds until meiosis is found. Success in finding meiosis in plants with one flower bud per inflorescence or many synchronous buds requires a knowledge of the life history of the plant. For example, members of the genus *Trillium* L. have one flower formed within each rhizome by the end of summer. *Trillium* must be located and marked in the field during flowering in the spring, and the rhizomes collected in late summer once the aboveground portion of the plant has died back. By collecting time in the autumn in Southeastern Ohio, the stakes for marking locations generally are covered with *Toxicodendron radicans* (L.) Kuntze.

Plants that I have found useful for classroom studies of meiosis are discussed in the following paragraphs. If plants are to be collected from the wild, it is important to be aware of federal, state and local laws about collecting plants, and to use wise collecting practices so that natural populations are not harmed. If purchasing wild flowers from commercial suppliers, check to make sure that the supplier is not collecting from the wild.

Mayapple My favorite from the standpoint of bivalent size and structure is Mayapple (*Podophyllum peltatum* L., Berberidaceae): the chromosomes are large enough to view easily with a 40x objective, and a relatively low haploid number (n = 6) facilitates the making of slides where the individual bivalents are seen clearly without many overlaps (Figures 1–8). In areas where Mayapple is common, buds may be collected just as the shoots emerge from the soil early in the Spring. (In Southeastern Ohio this is early-mid April.) Because there are many anthers per bud, and meiosis is not synchronous in the flower, when one flower bud of Mayapple is located with meiosis, most stages of meiosis can be found within the same flower.

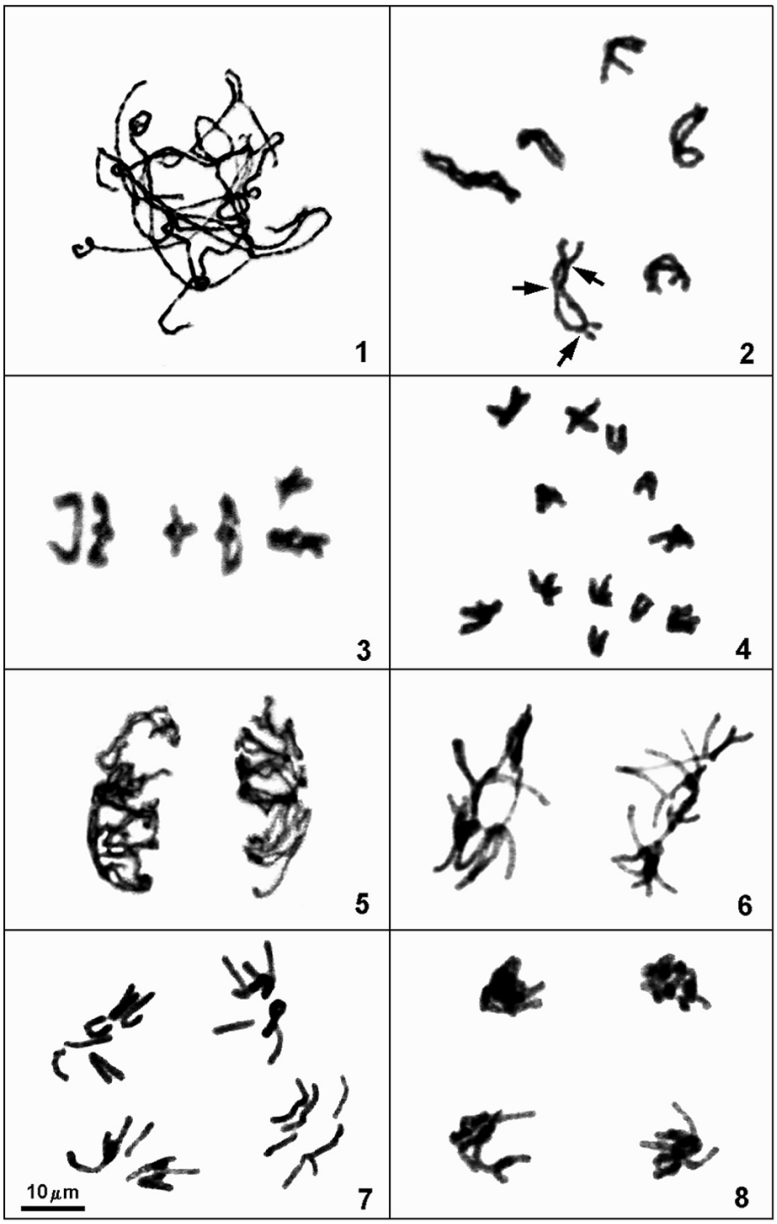

Plate 1, Figures 1–8. Feulgen squashes of meiosis in Mayapple (*Podophyllum peltatum*). Figure 1. Pachytene. **Figure 2.** Diplonema, with chiasmata (arrows) evident. **Figure 3.** Metaphase I with six bivalents. **Figure 4.** Anaphase I. **Figure 5.** Prophase II. **Figure 6.** Metaphase II. **Figure 7.** Anaphase II. **Figure 8.** Telophase II.

Plate 2, Figures 9 & 10. Comparison of mitosis and meiosis in Feulgen squashes of rye (*Secale cereale*). Figure 9. Colchicine-metaphase of mitosis in root cell showing 14 chromosomes. **Figure 10.** Metaphase I of meiosis with seven bivalents.

Grasses Several members of the grass family (Poaceae) are suited for meiotic studies: these include rye (*Secale cereale* L.) (Figures 9, 10) and bread wheat (*Triticum aestivum* L.), both of which have flowers arranged in spikes (Bennett & Smith 1972).

A major advantage of using either wheat or rye is that enough material for a class of 30–40 can be grown on one greenhouse bench. Select a spring cultivar so that vernalization is not required for flowering to occur. Seeds can be germinated on moist filter paper in petri dishes, and transplanted, one seedling per pot, to 10–12 cm diameter clay or plastic flowerpots. With continuous illumination in a greenhouse, plants with stages of meiosis develop in approximately 8–12 weeks, depending on the cultivar.

A disadvantage of using grasses for meiosis is that flowers (florets) are small, and some experience and practice are required to dissect anthers from the florets. A section on modified floral structure prior to meiosis is recommended if grasses are used. Meiosis occurs prior to emergence of the spike from the sheath of the flag leaf, so it is important to have a lot of material to gain practice in dissections of inflorescences and location of meiosis. Both rye and wheat generally have two florets per spikelet, so if tetrads or young microspores are found in the larger floret within a spikelet, the early stages of prophase I may be in the smaller floret.

Tradescantia Several horticultural strains of *Tradescantia* L. ("spiderwort," Commelineaceae) are useful for locating meiosis because there is a gradation of flower maturity within each inflorescence, and the bivalents are large enough to be observed easily with the 40x objective of a compound microscope (Figures 11, 12). Some cultivars of *Tradescantia* are tetraploid instead of the standard diploid, 2n = 12. We keep a population of *T. ohioensis* in our garden, and in the Spring and Summer periodically fix inflorescences for use in meiosis laboratories in November.

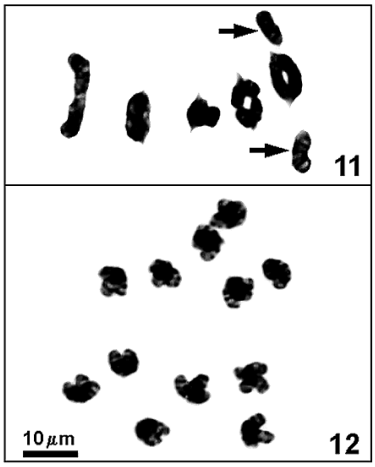

Plate 3, Figures 11 & 12. Feulgen squashes of meiosis in spiderwort (*Tradescantia* sp.). Figure 11. Metaphase I with five bivalents and two univalents (arrows). **Figure 12.** Anaphase I with six chromosomes in each complement.

Trillium Members of the genus *Trillium* (Liliaceae) have large, easily recognized, metaphase I bivalents and anaphase I chromosomes that show coiling (Figures 13, 14). *Trillium* species, however, do not have telophase I, interkinesis, and prophase II; instead meiosis goes directly from anaphase I to metaphase II. We have used *T. grandiflorum* L. because it is available locally, and goes through meiosis in flower buds formed in rhizomes in the fall. A classic paper for plant cytogenetics (Sparrow & Sparrow 1949) details how to store *T. erectum* L. just above freezing temperatures to prolong meiosis over several months.

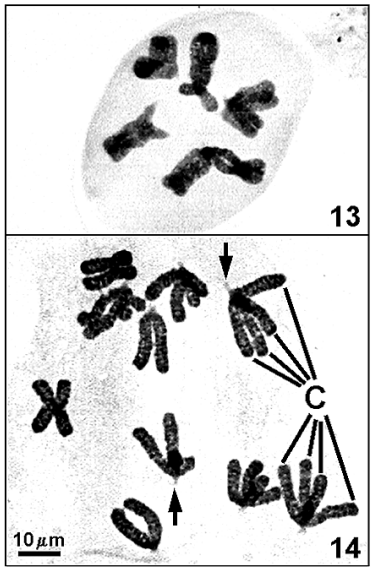

Plate 4, Figures 13 & 14. Orcein squashes of meiosis in *Trillium grandiflorum*. Figure 13. Metaphase I with five bivalents. **Figure 14.** Anaphase I with five chromosomes in each compliment, with chromatids (C) and centromeres (arrows) clearly evident.

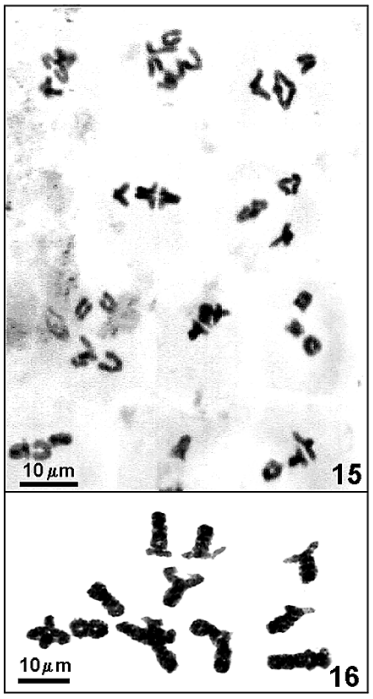

Plate 5, Figures 15 & 16. Orcein squashes of meiosis in *Ornithogalum virens* and *Lilium candidum*. Figure 15. Survey of group of meiocytes of *Ornithogalum virens*, showing three bivalents in metaphase I and several cells with early stages of anaphase I. **Figure 16.** Metaphase I of *Lilium candidum* showing 12 bivalents.

Ornithogalum Another genus in the Liliaceae suited for meiotic studies, *Ornithogalum* L., has flowers much smaller than lilies (next paragraph), but the inflorescences have gradation of floral stages that facilitate locating flowers with meiosis. Cultivars of *O. caudatum* Ait. ("sea onion") marketed for use as houseplants may have chromosome numbers of either n = 9 or 18. If you can find a cultivar of *O. virens* Lindl. (which also is sometimes sold as "sea onion") with n = 3 (Lima-de-Faria et al. 1959) (Figure 15), preparations of meiosis may be made with a minimum chance of overlapping of bivalents. Although *O. caudatum* is listed in several houseplant guides, one should be careful handling cut stems since the juice contains crystals that cause skin irritation. The relatively common, naturalized garden relative, *O. umbellatum* L. ("Star-of-Bethlehem"), also may be used, but there are only a few flowers per inflorescence and locating meiosis is more tedious than with either *O. caudatum* or *O. virens*.

Lilies Easter lily (*Lilium longiflorum* Thunb.) and Madonna lily (*L. candidum* L.) (Figure 16) have several large flower buds in an inflorescence. Although Shull & Menzel (1977) reported that stages of meiosis and bud length are not precisely correlated in lilies as was reported earlier (Erickson 1948), length of flower buds can be used to locate meiosis relatively easily. The flower buds are approximately 15–20 mm long when meiosis occurs, which makes dissecting anthers from them much easier than with some of the other plants such as grasses. Lilies can be grown in the garden and buds fixed for later use. Or, if greenhouse space is available, bulbs can be forced as is done commercially for Easter lilies.

Microscopic Procedures

Prior to starting the laboratory on meiosis, it is important to instruct students that some of the materials used in the procedures are potentially dangerous. Glacial acetic acid and the fixative should be handled in a well-ventilated area, preferably by a fume hood, and kept from skin and eyes. Students similarly must prevent staining reagents from coming in contact with skin or clothing. Any spills of staining reagents should be cleaned with soap and a little household bleach, thoroughly rinsed with water, and dried with paper towels. When using either dry ice or liquid nitrogen to make slides permanent, remind students of possible burning and blistering if either contacts skin.

Fixation & Storage

Either fresh or fixed material may be used in the orcein procedure detailed below. If fresh material is used for orcein squashes, however, the material must be fixed before further processing with the Feulgen procedure. Fix flower buds overnight in 3/1 (absolute ethanol/glacial acetic acid) at 4° C. Use enough fixative so that water from the inflorescences does not dilute the fixative and reduce its effectiveness. For example, when fixing spikes of rye or wheat, use at least 15 ml of fixative per

spike. Material may be stored in fixative for up to a week in the refrigerator. For longer storage, replace fixative with several changes of 70% ethanol and store in the freezer.

Orcein Squashes

For many plants, aceto-orcein may stain chromosomes as well as or better than with the Feulgen procedure. In those situations, the aceto-orcein procedure becomes the method of choice and there is no need to proceed to Feulgen staining. Some examples of orcein-stained materials that were made permanent with the quick freeze method are shown in Figures 13–16. Other plants may require that once temporary squashes with aceto-orcein staining have been used to locate flower buds with meiosis, the remaining anthers in the buds can be processed with the Feulgen procedure.

If fresh material is used, transfer the inflorescence into a petri dish or watch glass and cover it with distilled water. Cover fixed inflorescences with 70% ethanol. With the aid of a dissecting microscope, dissecting needles, and (or) fine forceps, carefully remove one anther from a flower.

For small anthers, whether fixed or fresh, transfer one entire anther to a drop of stain on a microscope slide, place a coverslip over the anther, tap it once with a dissecting probe or ballpoint pen to burst the anther, and evenly and gently press on the coverslip to flatten the cells. The sporocytes should be dispersed in an area around the wall material of the anther. Many beginners with meiotic material from anthers confuse tapetal nuclei dispersed among the meiocytes as meiotic nuclei, especially if the tapetum is of the binucleate type (Franceschi & Horner 1979). For some plants such as cereals, premeiotic sporocytes and cells in early stages of meiosis may remain together in columns.

For fresh material of plants with large anthers such *Lilium*, *Trillium* or Mayapple, grasp the anther between thumb and forefinger, cut off the tip of the anther with a razor blade or scalpel, squeeze the contents into a drop of stain on a microscope slide, and lower a coverslip over the specimen. Fixed large anthers must be cut into several pieces while in the drop of stain before adding the coverslip. Lightly tap the coverslip with a dissecting probe to break up large clumps of cells, cover the slide with several layers of paper towel or filter paper, evenly and gently press on the coverslip to flatten the cells, and examine the preparation for meiosis.

Microsporocytes are delicate, so it is easy to put too much pressure on the coverslips during either tapping on the coverslip to break up clumps of cells or pressing to flatten the cells. Doing either will burst the sporocytes and produce "chromosomal soup," or flatten the cells and chromosomes beyond recognition. Experience is the only teacher for getting the right touch.

If meiosis has been completed and you see microspores or pollen, discard the remaining portion of the flower and repeat with the next smaller flower. If the microsporocytes are too young, repeat the procedure with the next larger flower. Once a flower with a meiotic stage is located, remove the remaining anthers from the flower for staining with aceto-orcein, or for processing with the Feulgen procedure.

Feulgen Procedure

If fixed material was used to locate buds with meiosis, go to the following paragraph. If fresh flower buds were used for the orcein procedure, transfer the remaining anthers from selected flower buds to 3/1 fixative and allow to fix in the refrigerator overnight. Once material has been fixed, it may be stored in 70% ethanol in the freezer.

Rinse fixed anthers, whether they were in 3/1 fixative or stored in 70% ethanol, three times with distilled water, 5 minutes each. Make sure that the anthers sink in the last change of distilled water. Sometimes anthers must be helped to sink by dropping distilled water directly onto them from a pipet.

Decant the distilled water and replace it with 1 N HCl at 60° C. Hydrolyze the anthers in 1 N HCl for 10 minutes at 60° C. Leave the cap of the vial off during hydrolysis. Stop the hydrolysis by adding ice-cold distilled water to the vial, pipet off all of the diluted HCl within 60 seconds of adding the distilled water, and replace with just enough Schiff reagent to cover the anthers.

Allow the material to stain in Schiff reagent for 1/2–1 hour at room temperature or until the anthers turn a deep magenta. Keep the cap of the vial on during staining. Material may be kept in Schiff reagent overnight in the refrigerator.

Transfer an anther to a drop of 45% acetic acid on a clean microscope slide and gently macerate the anther with a fine scalpel blade, dissecting probe, or fine forceps. Lower a coverslip over the specimen, cover the slide with filter paper or paper towel, evenly press (very little pressure is required) to flatten the cells, and examine for meiotic stages. Make the slide permanent with the quick freeze method.

Modifications of the Protocol

Once anthers have been stained in Schiff reagent, they may be dehydrated for long-term storage in the freezer. Replace the Schiff reagent with 70% ethanol for 15 minutes. The ethanol will turn magenta. Replace with 95% ethanol for 15 minutes, and follow with two changes, 15 minutes each, of 100% ethanol. The ethanol will continue to turn light magenta or pink. Store anthers in a third change of 100% ethanol in the freezer for overnight. The next day replace with fresh 100% ethanol and store indefinitely in the freezer. If the ethanol turns pink, replace it. The intensity of the Schiff reagent will increase if anthers are prepared this way. Also, the anthers are hardened by the ethanol treatment and microsporocytes consequently may not burst as easily when being squashed. Anthers that are stored in 100% ethanol should be placed in 45% acetic acid for 1–3 minutes at room temperature before being squashed in 45% acetic acid.

Quick Freeze Method for Making Slides Permanent

The original procedure described by Conger & Fairchild (1953) was based on the freezing of freshly prepared slides with dry ice. Many people today, however, use liquid nitrogen if it is available. Occasionally liquid nitrogen causes slides to crack when they are plunged into it.

1. Freeze slide (coverslip up) on a block of dry ice, or by plunging it into liquid nitrogen.
2. Pop off the coverslip with a razor blade.
3. Immerse the slide in 95% ethanol for approximately 1 minute.
4. Remove the slide from the ethanol and cover material with a clean coverslip and either Diaphane™ or Euparal™ mounting resin. If a xylene- or toluene-soluble resin is to be used (e.g. Permount™), the slide must be passed through three changes of 100% ethanol, one change of 1/1 ethanol/xylene (or toluene), and two or three changes of dry xylene (or toluene) before mounting with the resin. A limonene-based clearing agent marketed by Fisher Scientific, Hemo-De™, is a suitable replacement for xylene or toluene.
5. Although slides may be examined immediately after applying a coverslip with mounting resin, the

mounting resin may not become entirely hardened for several months. Use caution and a minimum amount of pressure if the coverslip must be cleaned. Slides should be stored flat in a dust free environment until the mounting resin has hardened sufficiently to prevent easy movement of the coverslip.

Discussion

Having students prepare their own slides provides them with a sense of accomplishment. There will be, however, several students within a laboratory who are not successful in preparing slides of meiosis, and for those students it is important to be supportive of their efforts and to encourage them to examine slides of their peers. I generally treat the meiosis laboratory as a group project: Few students will locate every stage of meiosis, but collectively the research group will produce a set of slides with all major stages.

By observing slides that their classmates and they have made, students are able to directly observe differences between meiotic and mitotic chromosomes (Figures 9, 10). Students also actively follow how cells with haploid nuclei are formed from one cell with a diploid nucleus. Although the static images of meiosis do not directly give the duration for prophase I when homologous chromosomes pair (synapsis) (Figure 1), because prophase I stages are generally found by students more often than later stages of meiosis, this should indicate to them that prophase I is longer than the other stages. Similarly, by observing slides they have made, students pay attention to late prophase I bivalents which can be seen to consist of two chromosomes attached at chiasmata, the cytological indicators of crossing over (Figure 2). By counting the haploid number of metaphase I bivalents (Figure 3), and observing anaphase I (Figure 4) homologous chromosomes separated and oriented toward opposite poles, students directly see how chromosomal numbers are reduced. Also, how chromatids in anaphase I repel each other, but are held together by the centromeric regions (Figures 4, 12, 14), illustrates differences between mitotic and meiotic chromosomal structure. Slides that show telophase I, interkinesis, and prophase II of diads (Figure 5), introduce how the second meiotic division (Figures 6–8) superficially appears similar to mitosis. Discussions could be held to emphasize that division II, unlike mitosis, does not have synthesis of DNA during the preceding interkinesis, and chromatids within a given chromosome may not be identical due to crossing-over in prophase I. Each of the haploid cells in a tetrad becomes a genetically unique microspore, the first cell in the gametophytic generation.

Because the immediate meiotic products in plants are spores as opposed to gametes in higher animals, the use of flowering plants to examine meiosis leads naturally into considerations of alternation of generations in plant life cycles and how plants can be used to understand fundamental developmental processes (Goldberg 1988). Microscopic examination of floral products also serves as a launch into pollen and its role in the life cycle of seed plants. More in-depth topics that follow introductory examination of meiosis may include role of the tapetum in microsporogenesis of angiosperms, comparison of seed development in gymnosperms and angiosperms, fruit development in angiosperms, control of flowering in angiosperms, pollination mechanisms, comparison of seed plants to non-seed plants, and cytogenetics of the breeding of crop plants.

Formulary

Schiff Reagent Schiff reagent may be purchased from Sigma Chemical Company (P.O. Box 1458, St. Louis, MO 63178), or prepared by the Lillie (1951) method. Add 1 g of basic fuchsin and 1.9 g sodium metabisulfite to 100 ml of 0.15 N HCl. Stir for 2 hours. The solution should be brown. Add 0.5 g activated charcoal, shake for 2 minutes, and filter. Solution should be light straw color or clear. Store in a dark bottle in a refrigerator.

Aceto-Orcein Use either natural or synthetic orcein. Ignore what you might read in some protocols about heating or boiling in preparation of aceto-orcein. Add 0.5 g to 100 ml of 45% acetic acid and let stand for several days to several weeks at room temperature. The stain improves with age, and may continue to improve over several years. Filter before use. An alternative is to use propionic acid in place of acetic acid. This works just about as well and does not have the strong smell of acetic acid. Some people use a one-to-one mixture of lactic acid and either acetic or propionic acid (add the acids together, then make to 45% with distilled water).

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